

From the Neurosurgical Research Department, University of Lund,
Lund, Sweden

Central catecholamine pathways Involved in cerebral vasospasm and Changes in cerebral blood flow and Glucose metabolism after a Subarachnoid haemorrhage

An experimental study in the rat

Tia Delgado

Lund 1986

From the Neurosurgical Research Department, University of Lund,
Lund, Sweden

Central catecholamine pathways Involved in cerebral vasospasm and Changes in cerebral blood flow and Glucose metabolism after a Subarachnoid haemorrhage

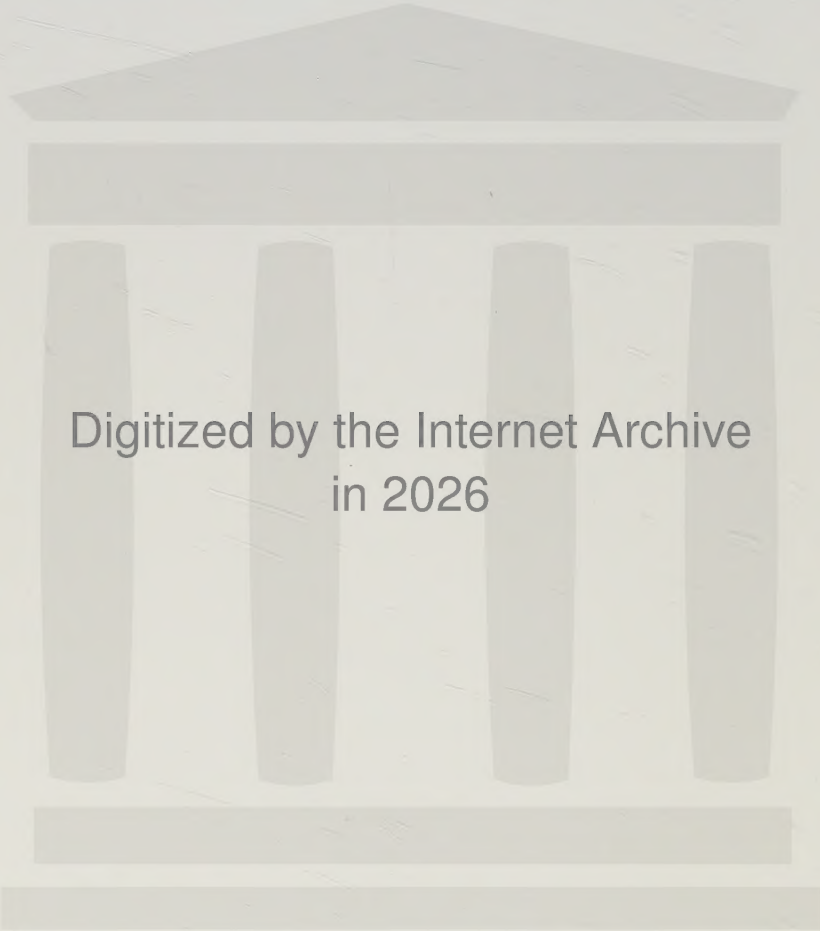
An experimental study in the rat

Tia Delgado



Lund 1986

To my mother



Digitized by the Internet Archive
in 2026

https://archive.org/details/IA41552414_0007

The thesis is based on the following papers, which will be referred to by their Roman numerals:

- I. Delgado TJ, Brismar J, Svendgaard NAa (1985) Subarachnoid haemorrhage in the rat: Angiography and fluorescence microscopy of the major cerebral arteries. *Stroke* 16:595-602.
- II. Delgado TJ, Arbab MA-R, Diemer NH, Svendgaard NAa (1986) Subarachnoid haemorrhage in the rat: Cerebral blood flow and glucose metabolism during the late phase of cerebral vasospasm. *J Cereb Blood Flow Metabol*, submitted for publication.
- III. Svendgaard NAa, Brismar J, Delgado TJ, Rosengren E (1985) Subarachnoid haemorrhage in the rat: Effect on the development of vasospasm of selective lesions of the catecholamine systems in the lower brain stem. *Stroke* 16:602-608.
- IV. Delgado TJ, Diemer NH, Svendgaard NAa (1986) Subarachnoid haemorrhage in the rat: Cerebral blood flow and glucose metabolism after selective lesions of the catecholamine systems in the brain stem. *J Cereb Blood Flow Metabol*, submitted for publication.
- V. Svendgaard NAa, Delgado TJ, Brun A (1986) Effect of selective lesions in the hypothalamic-pituitary region on the development of cerebral vasospasm following an experimental subarachnoid haemorrhage in the rat. *J Cereb Blood Flow Metabol*, submitted for publication.

CONTENTS

Introduction	9
Objectives	16
Methods	16
Results and discussion	22
Conclusions	28
Acknowledgements	30
References	31
Paper I	45
Paper II	63
Paper III	89
Paper IV	107
Paper V	127

INTRODUCTION

Cerebral vasospasm is an angiographically demonstrable, variable narrowing of the major intracranial arteries that can be clinically asymptomatic or give rise to increasing neurological deficits or death. Vasospasm is most commonly seen following a subarachnoid haemorrhage (SAH) due to rupture of an intracranial aneurysm. However, it can also occur after head trauma (Wilkins and Odom 1970; Suwanwela and Suwanwela 1972; MacPherson and Graham 1978) and after intracranial surgery in the hypothalamic-pituitary area (Kågstöm et al. 1969; Simeone and Peerless 1975; Peerless 1980). Spasm develops in 40 to 70% of patients with aneurysmal rupture (DuBoulay 1963; Allcock and Drake 1965; Weir et al. 1975, 1978; Kassell et al. 1985) and in 5 to 10% of patients with head trauma (Wilkins 1975). About one half of the patients with spasm following aneurysmal SAH develop signs of cerebral ischaemia or die (Saito et al. 1977; Sano and Saito 1978; Sundt et al. 1982). The sequelae of vasospasm after head trauma or intracranial surgery have been less well investigated.

Background

In 1949, Robertson suggested that the delayed ischaemic complications seen after aneurysm rupture might be secondary to vasospasm. The angiographical appearance of cerebral vasospasm was first described in 1951 by Ecker and Riemenschneider. Subsequently, Kågstöm et al. (1966) demonstrated that angiographical spasm following a SAH is delayed and progressive, reaching a maximum several days after the bleed. Later studies confirmed that the delayed and clinically deleterious spasm reaches a maximum about seven days after the SAH (Bergvall and Galera 1969; Bergvall et al. 1973; Weir et al. 1978). Experimentally, vasospasm has been described as biphasic with a short-lived acute phase and a late phase reaching a maximum between day two and day seven post SAH, depending on the species used (Brawley et al. 1968; Kågstöm et al. 1969; Kuwayama et al. 1972; Nagai et al. 1974; Barry et al. 1979; Peerless et al. 1982).

In 1965, Echlin demonstrated that the direct application of blood to the cerebral arteries in animals induced a constriction. Subsequent studies confirmed the ability of blood per se to produce arterial

constriction (Allcock 1966; Chow et al. 1968; Kapp et al. 1968; Landau and Ransohoff 1968). The relationship between blood and vasospasm is further supported by the good correlation between the amount of subarachnoid blood and the degree of spasm in both clinical and experimental studies (Fisher et al. 1980; Mizukami et al. 1980a; Espinosa et al. 1982). Also, the use of antifibrinolytic treatment which delays the dissolution of blood clots increases the frequency and probably also the severity of vasospasm (Kågström and Palma 1972; Kassell et al. 1984).

Since the observation of Echlin in 1965, numerous investigations of vasospasm have been carried out, particularly in the acute phase after intracisternal blood injection or after application of blood or blood components to cerebral arteries in vitro (Fraser et al. 1970; Allen et al. 1974a; Kapp et al. 1968; Nagai et al. 1974; Endo and Suzuki 1977; Brandt et al. 1979; Toda et al. 1980; Wellum et al. 1980; Quintana et al. 1982; Voldby et al. 1984; White and Robertson 1985). These studies provide information about short-lived arterial constriction, but are not ideal for the study of the late prolonged type of vasospasm. Difficulties in the development of experimental SAH models to investigate late vasospasm have been the lack of reproducibility of the late spasm and the failure of most species to develop delayed neurological deficits post SAH. The latter is a serious drawback in therapeutic models, but is of minor importance if the objective is the investigation of the basic mechanisms behind vasospasm. Recently, the use of repeated cisternal blood injections or the direct application of blood clots to the major cerebral arteries has lead to an increased reproducibility of late experimental angiographical vasospasm (Varsos et al. 1983; Espinosa et al. 1984a; Sahlin et al. 1986). However, the mechanisms behind vasospasm remain unknown. Thus, it has not been determined if vasospasm represents a prolonged contraction of the smooth muscle cells of the cerebral arteries, a failure of relaxation of these cells or a pathological thickening of the vessel wall.

Proponents of the contraction theory have generally focused on the identification of a spasm-inducing factor contained in blood or liberated from blood clots into the cerebrospinal fluid (CSF). It has also been suggested that tissue damage produced by the SAH could release vasoconstrictive substances (White et al. 1975). Vasoactive substances suggested as spasmogens include norepinephrine (Kapp

et al. 1968; Alksne and Greenhoot 1974), serotonin (Raynor et al. 1961; Chow et al. 1968; Allen et al. 1974a,b), angiotensin (Kapp et al. 1968; Allen et al. 1974c), histamine (Allen et al. 1974c, White et al. 1975), thrombin or plasmin (White et al. 1980), fibrin degradation products (Forster et al. 1980), thromboxane A_2 (Ellis et al. 1977) and hydroperoxides (Sasaki et al. 1980). Breakdown products of erythrocytes have also been suggested as the spasm-inducing factor (Osaka 1977; Ohta et al. 1980). However, the constriction produced by all of the aforementioned is generally short-lived. The CSF from SAH patients has been evaluated in both in vitro (Allen et al. 1974b; Boullin et al. 1976; Hunt et al. 1979) and in vivo studies (Raynor et al. 1961; Boullin et al. 1981; Brandt et al. 1981) and found to induce arterial constriction, although the origin and nature of the vasoconstrictive factor(s) in CBF remain unknown.

It has also been suggested that blood could induce arterial constriction via the mechanism of denervation hypersensitivity (Fraser et al. 1970; Peerless and Kendall 1975). Studies with experimental SAH models have shown both a depletion of catecholamines in perivascular nerves (Fraser et al. 1970; Peerless and Kendall 1975; Svendgaard et al. 1977; Lobato et al. 1980a) and an increased sensitivity to noradrenaline and serotonin (Svendgaard et al. 1977; Lobato et al. 1980b). However, therapeutic intervention with adrenergic blockade has not proven successful (Wilkins 1980).

Vasospasm as a failure of relaxation of the cerebral arteries has recently been investigated. It has been proposed that endothelial degeneration resulting from the SAH could decrease the synthesis of prostacyclin, a potent vasodilator normally produced by the endothelium (Boullin et al. 1979). Experimentally, a progressive decrease in prostacyclin synthesis post SAH has been demonstrated (Sasaki et al. 1981). Further, a decrease in prostacyclin synthesis could result in an imbalance in the prostacyclin/thromboxane A_2 ratio which has been suggested to control vascular tone (Sasaki et al. 1982; Chan et al. 1984). Thromboxane A_2 synthesized from platelets is a potent vasoconstrictor (Ellis et al. 1977). Blood surrounding the cerebral arteries might also impair the vasodilatory capacity of the arteries via hypoxia (Simeone and Vinall 1980) or via a direct effect of blood components on neurogenic vasodilation (Lee et al. 1984; Martin et al. 1985).

A number of investigators have suggested that the arterial nar-

rowing seen on angiography represents a structural and pathological change in the vessel wall induced by blood (Alksne 1974; Clower et al. 1980; Mizukami et al. 1980b). Histopathological changes including intimal thickening have been found in cerebral arteries from SAH patients (Conway and McDonald 1972; Alksne 1974; Hughes and Schianchi 1978; Clower et al. 1980; Mizukami et al. 1980b) and similar changes have been induced in experimental SAH models (Fein et al. 1974; Tanabe et al. 1978; Tani et al. 1978; Espinosa et al. 1984b). However, the time course for development of the morphological changes and the degree of vascular thickening generally seen, speak against this theory (Tanabe et al. 1978; Nagasawa et al. 1982; Espinosa et al. 1984b). Also, other investigators have not found the described morphological changes in either human or animal cerebral arteries post SAH (Mayberg et al. 1978; Eldevik et al. 1981; Liszczak et al. 1984).

Finally, it is of great interest theoretically, that cerebral vasospasm has been reported to occur in patients with head trauma without blood in the subarachnoid space (Wilkins 1970; Suwanwela and Suwanwela 1972), in patients with pheochromocytoma (Armstrong and Hayes 1961) or in patients with meningitis (Greitz 1964; Lyons and Leeds 1967). No definitive explanation for the occurrence of spasm in these patients has been offered.

Pathophysiology

Vasospasm has been generally accepted as a major cause of morbidity and mortality following SAH (Fletcher et al. 1959; Allcock and Drake 1965; Griffith et al. 1972; Weir et al. 1975; Saito et al. 1977; Sano and Saito 1978). Narrowing of the cerebral arteries increases the cerebrovascular resistance and can cause a decrease in cerebral blood flow (CBF) below critical levels resulting in ischaemia. Both global and regional reductions in CBF have been described in patients with SAH and in experimental studies (James 1968; Ferguson et al. 1972; Hashi et al. 1972; Heilbrun et al. 1972; Petruk et al. 1973; Grubb et al. 1977; Ishii 1979; Jakubowski et al. 1982; Mickey et al. 1984). However, a number of investigators have reported that the global reduction does not seem to be related to angiographical vasospasm (Ferguson et al. 1972; Heilbrun et al. 1972; Bergvall et al. 1973; Ishii 1979; Espinosa et al. 1984a; Mickey et al.

1984; Sahlin et al. 1986). A possible explanation for the global reduction in CBF is that the angiographically not visible, small intraparenchymal arteries or arterioles also are constricted. It is however, not likely that the arterioles participate in spasm since an increase in cerebral blood volume has been found in patients with severe diffuse vasospasm suggesting an arteriolar dilatation (Grubb et al. 1977; Martin et al. 1984). The global reduction has also been suggested to be secondary to a primary depression of metabolism induced by the SAH (Heilbrun et al. 1972). It has been shown in SAH patients that there is a decrease in the cerebral metabolic rate of oxygen ($CMRO_2$) associated with the decrease in CBF (Kutsuzawa et al. 1968; Grubb et al. 1977; Martin et al. 1984; Powers et al. 1985; Voldby et al. 1985a). Other pathophysiological manifestations of SAH such as intracranial hypertension, or the presence of haematomas or hydrocephalus might also play a role in the global flow reduction.

On the other hand, regional reductions in CBF in brain regions perfused by arteries in spasm have been frequently described (Ferguson et al. 1972; Heilbrun et al. 1972; Ishii 1979; Mickey et al. 1984). Also, there is a good correlation between severe angiographical spasm, neurological deficits, and signs of infarction (low density or "lucent" areas) on computerized tomography (CT) (Bryan et al. 1979; Saito et al. 1979; Voldby et al. 1985a). Additional data supporting the development of ischaemic changes/infarcts in areas supplied by spastic arteries has been presented in several neuropathological studies (Smith 1963; Crompton 1964; Graham et al. 1983). However, there can be a discrepancy between the angiographical findings and the neurological symptoms (Graf and Nibbelink 1974; Kelly et al. 1977). The discrepancy reflects the importance of other factors that interact with vasospasm and affect the cerebral perfusion. These factors include the adequacy of the collateral circulation, the perfusion pressure, and the CO_2 reactivity and autoregulatory capacity of the cerebral vessels. Simeone et al. (1972) showed that angiographical constriction is not necessarily associated with a significant decrease in regional CBF until there is at least a 50% reduction in vessel calibre and attributed this to the cerebral circulatory reserve capacity. Espinosa et al. (1984a) evaluated the collateral circulation angiographically in baboons with spasm, but normal CBF, and noted that it appeared to be maximal at the time point of maximal spasm. Both clinical (Pickard et al. 1980; Farrar et al. 1981; Voldby et al. 1985b)

and experimental (Boisvert et al. 1979; Pickard et al. 1979; Mendelow et al. 1981; Svendgaard et al. 1983) studies have shown that following a SAH, there is an impairment in autoregulation and CO₂ response that can adversely affect the cerebral perfusion. Both impairments are particularly important in the management of patients undergoing intracranial surgery for ruptured aneurysms in determining those patients at particular risk for developing ischaemic complications from vasospasm post-operatively.

Therapy

The vast number of treatments suggested for cerebral vasospasm reflects the lack of understanding of the basic mechanism (s) underlying the arterial constriction. The aims of treatment have been either to prevent or reverse the arterial narrowing or the prevention or reversal of ischaemic neurological deficits.

Numerous compounds have been evaluated for the prevention or reversal of the arterial constriction (Wilkins 1980; Kassell et al. 1985). Such agents include monoamine antagonists (Fraser et al. 1970; Nagai et al. 1975; Zervas et al. 1973, 1974, 1979) and agonists (Sundt et al. 1973; Flamm et al. 1975; Sundt 1980), prostacyclin (Boullin et al. 1979, Quintana et al. 1982; Fukumori et al. 1983), thromboxane A₂ synthetase inhibitors (Sasaki et al. 1982; Chan et al. 1984; Fukumori et al. 1984; Tani et al. 1984), angiotensin converting enzyme inhibitor (Gavras et al. 1981), anti-inflammatory agents (Fox and Yasargil 1975; Hashi et al. 1980; Chyatte et al. 1983; White and Robertson 1983) and free radical scavenging agents (Kato et al. 1985; Oba et al. 1985). None of these drugs have been conclusively shown to relieve or prevent vasospasm in patients.

Currently the drugs receiving most attention as spasmolytics are the calcium channel blocking agents (Hayashi and Toda 1977, Allen and Bahr 1979; Brandt et al. 1979; Kazda and Towart 1982; Tanaka et al. 1982). However, the calcium channel blocking agents do not appear to prevent or reverse prolonged vasospasm which could be due to the fact that the spasmogenic agent is mainly dependent on intracellular calcium (Barry et al. 1985). Although the calcium blocking agent, nimodipine, has been found not to relieve or prevent angiographical vasospasm (Grotenhuis et al. 1984; Ljunggren et al. 1984), it has been reported to decrease the occurrence of the ischae-

mic complications of spasm (Allen et al. 1983; Auer 1984; Ljunggren et al. 1984). Since it also has been found that oral or intravenous administration of nimodipine does not increase CBF following an experimental SAH (Espinosa et al. 1984a; Nosko et al. 1985; Sahlin et al. 1986), the beneficial effect of nimodipine may instead be exerted at the neuronal level by a direct prevention of ischaemic cell death (Trump et al. 1980; Hass 1981).

Another interesting approach in the treatment of spasm is to prevent or reverse the activation of the actin-myosin complex by calmodulin. So far the studies with calmodulin antagonists have been promising, but more investigations are needed (Blaumanis and Grady 1982; Nakano et al. 1984).

Further treatment for the prevention of spasm is the removal of the blood clots from the basal cisterns either mechanically (Sano and Saito 1980; Mizukami et al. 1982) or with fibrinolytic therapy (Peterson et al. 1980; Shiobara et al. 1985). At present, there is a tendency to be less aggressive in the surgical removal of blood clots due to the risk of damaging brain parenchyma or small perforating vessels. In addition, doubts concerning the efficacy of the method in decreasing the frequency and degree of vasospasm have been reported (Kassell et al. 1985). Theoretically, the fibrinolytic therapy is interesting but it is so far at an experimental stage.

Difficulties in relieving vasospasm have resulted in attempts to prevent or reverse neurological deficits through an improvement in the haemodynamic state of SAH patients by changing the rheological characteristics of blood, increasing the CBF, or improving oxygen delivery to the brain. Drugs improving the rheological status by decreasing blood viscosity include dextran (McMurty et al. 1967; Wood et al. 1982), mannitol (Little 1978; Suzuki and Yoshimoto 1979), albumin (Little et al. 1981) and heparin (Wilkins 1980). Calcium blocking agents can also lead to improvement via their effect on platelet aggregation (Dale et al. 1983).

The combination of blood volume expansion and induced hypertension has been used at a number of clinics to increase CBF in patients at risk for developing neurological deficits (Kindt et al. 1980; Ritchie et al. 1980; Kassell et al. 1982). Ischaemic deficits can be reversed by this therapy, although it is technically demanding and may be complicated by a number of problems including pulmonary edema (Kassel et al. 1982).

Finally, the oxygen delivery can be improved with fluorinated hydrocarbons (Handa et al. 1983) either alone or combined with hyperbaric oxygen (Heyman et al. 1966).

OBJECTIVES

The occurrence of vasospasm not only following a subarachnoid haemorrhage but in head trauma patients without blood in the subarachnoid space and in patients with pheochromocytoma lead us to develop a model suitable for investigating if a central mechanism and in particular the central monoamine systems could underlie the development of spasm. The objectives were as follows:

1. To design a simple and inexpensive small animal SAH model giving a reproducible angiographical vasospasm and allowing evaluation with CBF and glucose metabolism (CMRglu) studies (paper I and II).
2. To evaluate via selective lesions in the mesencephalon or medulla oblongata if the central noradrenergic systems are involved in the development of angiographical spasm (paper III) or the post SAH changes in CBF and glucose metabolism (paper IV).
3. To determine via selective lesions in the hypothalamic-pituitary area, if that region contains a site coresponsible for vasospasm (paper V).

MATERIAL AND METHODS

Animals

The experiments were performed on adult, male Sprague-Dawley rats (SPF strain, Møllegaards Avslaboratorium, Køge, Denmark). All animals had free access to rat pellets and water with the exception of those scheduled for CBF and CMRglu studies. The latter were fasted for 12-18 hours before the studies.

SAH model

After anaesthetizing the animals with chloral hydrate (250 mg/kg i.p.), an opaque X-ray catheter was inserted into the cisterna magna for injection of blood. Three to seven days later, 0.07 or 0.3 ml of homologous blood was injected via the subcutaneous catheter,

and the animals evaluated with angiography, fluorescence microscopy or autoradiographic CBF and CMRglu studies.

Lesions

Lesions were made 9-28 days prior to the angiography or CBF and CMRglu measurements. Sham lesions were made as appropriate. Stereotaxic or systemic lesions were produced in animals anaesthetized with Brietal (Lilly, 40 mg/kg i.p.). Surgical lesions were produced in intubated animals anaesthetized with halothane and artificially ventilated with a 70% nitrous oxide and 30% oxygen mixture.

Analytical techniques

1. Blood gases and pH

Anaerobically obtained arterial blood samples of 150-200 μ l were measured using a pH-blood gas analyzer 413, Scandiametric.

2. Glucose

Plasma glucose was determined with a glucose oxidase method using a Greiner G300.

3. Noradrenaline (NA)

Noradrenaline was determined in brain tissue using liquid chromatography with electrochemical detection (Eriksson and Persson 1982).

Angiography

Surgical preparation.

Anaesthesia was initiated with 4% halothane. The animals were intubated, and artificially ventilated with a 70% nitrous oxide and 30% oxygen mixture. Anaesthesia was maintained with 0.75% halothane. Catheters were inserted into the axillary artery bilaterally for subsequent angiography. The femoral artery and vein were cannulated for continuous blood pressure monitoring, infusion of drugs or blood sampling. Heparin (Vitrum, 75 IU/kg) was injected intravenously. Following the surgical procedure, the halothane was switched

off. Suxamethonium chloride (Celocurin, Vitrum; 3 mg/kg i.v.) was given and at least one half hour was allowed to pass before the angiography. Blood samples were drawn to evaluate the pH, PaO₂ and PaCO₂.

Angiographic procedure.

Vertebro-basilar angiography was performed via bilateral axillary catheters. Metrizamide (Amipaque®, Nyegaard and Co., Oslo, Norway) was used as the contrast medium. Following the control angiography, homologous blood was injected via the subcutaneous catheter. Repeat angiography was performed at predetermined time points following the SAH. Measurements of vertebral and basilar arteries were made using a technique similar to that described by Gabrielsen and Greitz (1970). The diametres at four pre-selected points within the vertebro-basilar system were averaged and expressed as a percentage of the control values.

Fluorescence microscopy.

The cerebral vessels were dried in vacuo in a dessicator over phosphorous pentoxide for at least two hours. They were then exposed to formaldehyde gas of optimal humidity at 80°C for one hour (Falck et al. 1962; Björklund et al. 1972). The brain stem samples were freeze-dried and were reacted similarly using paraformaldehyde equilibrated at 50% humidity. The preparations were mounted in liquid paraffin and examined in a Zeiss fluorescence microscope.

Autoradiographic CBF and/or CMRglu studies

Surgical preparation.

The anaesthesia was as described for the animals prepared for angiography. Both femoral arteries were cannulated for blood pressure monitoring and for blood sampling. Catheters were inserted into the femoral veins for injection of drugs and later infusion of the tracers. Heparin (Vitrum, 75 IU/kg) was given intravenously. The halothane was switched off with completion of the surgery. Suxamethonium chloride (Celocurin, Vitrum; 3 mg/kg i.v.) was given and at least one half hour was allowed to pass before the CBF/CMRglu studies. Blood samples were drawn to evaluate the blood gases and the haematocrit was measured.

Local CBF.

Local CBF was measured according to the technique described by Sakurada et al. (1978) as modified by Gjedde et al. (1980). One arterial catheter was connected to a constant velocity withdrawal pump for mechanical integration of tracer concentration. The tracer ^{14}C -iodoantipyrine was given as an intravenous bolus and arterial blood was withdrawn with the pump over 20 seconds. Immediately after the last sample, the animal was decapitated and the brain removed and frozen in isopentane chilled to -50°C . The ^{14}C activity of the blood samples was determined with beta-scintillation counting.

Local CMRglu.

Local CMRglu was determined using the method described by Sokoloff et al. (1977). The tracer ^{14}C -deoxyglucose was infused intravenously over 30 seconds and fifteen samples of arterial blood were taken at preselected time points over 45 minutes. The blood samples were immediately centrifuged and the plasma separated for determination of ^{14}C -deoxyglucose by beta-scintillation counting and for determination of glucose concentration. After the last sample, the animal was decapitated and the brain frozen as described.

Simultaneous local CBF and CMRglu.

Local CBF was measured according to the technique of Gjedde et al. (1980) while local CMRglu was measured using the method presented by Sokoloff et al. (1977), substituting ^3H -deoxyglucose for ^{14}C -deoxyglucose. ^3H -deoxyglucose was infused intravenously over 30 seconds. Arterial blood samples were taken and processed as described over 45 minutes. After the last sample, one arterial catheter was connected to the constant velocity withdrawal pump. ^{14}C -iodoantipyrine was given as an intravenous bolus and arterial blood was withdrawn with the pump over 20 seconds. The animal was decapitated and the brain frozen as described. The concentration of ^{14}C and ^3H in the plasma and blood samples was measured using beta-scintillation counting with programs for quench correction and double label counting.

Lumped constant (LC).

The anaesthesia and cannulation procedures in the animals used to evaluate the LC were as described for the CBF and/or CMRglu studies. ^{14}C -deoxyglucose and ^3H -3-0-methylglucose were infused as an intravenous injection. Arterial blood samples were taken during ten minutes for determination of the plasma deoxyglucose and 3-0-methylglucose. Immediately after the last sample, the animal was decapitated and the brain frozen.

Autoradiographic procedures.

The ^{14}C or ^3H activity in the brain tissue was determined after sectioning the brain in 20 μm sections at -20°C . The sections for detection of ^{14}C activity were processed for autoradiography using X-ray film (Kodak-MIN-R) and 16 days exposure time. The sections were placed on film together with a set of calibrated ^{14}C standards. Brain sections from animals receiving both ^{14}C -iodoantipyrine and ^3H -deoxyglucose were processed as follows: The sections for detection of ^3H deoxyglucose content were washed immediately after cutting, 2x1 minute in 2.2 dimethoxypropane (DMP) which solubilizes the iodoantipyrine without removing deoxyglucose (Diemer and Rosen n 1981 ; Gjedde and Diemer 1985). The sections were placed on ^3H sensitive film (LKB Ultrofilm) with a set of standards calibrated for ^3H and an exposure time of 19 days was used. Neighboring brain sections were placed on X-ray film (Kodak MIN-R) containing a protective gelatin coating that absorbed the ^3H radiation without affecting the ^{14}C . A set of calibrated ^{14}C standards were placed on the film with the brain sections.

In the animals receiving ^{14}C -deoxyglucose and ^3H -3-0methylglucose the brain sections were processed similarly except that the sections were not washed in 2.2 DMP.

Autoradiograms were analyzed using the memory of a Leitz TAS plus (R) image analyzer. Brain regions were encircled using a light pen and the average optical density obtained. The computer converted optical density to ^{14}C or ^3H activity using curves determined from the calibrated standards.

Calculations.

CBF.

Local CBF was calculated in the single or double isotope animals using the equation of Gjedde et al. (1980):

$$f^{bl} = \frac{C_{Br}(T)}{E(T) \int_0^T C_a(t) dt}$$

where f^{bl} is the blood flow per unit mass, $C_{Br}(T)$ the isotope content, $E(T)$ the net extraction fraction of the isotope in the time from $t=0$ to $t=T$. t = the variable time and T = the experimental time (20 seconds). $C_a(t)$ is the arterial blood concentration of the isotope at time t .

CMRglu.

Local CMRglu was measured according to the equation of Sokoloff et al. 1977:

$$R_i = \frac{C_i^*(T) - k_1^* e^{-(k_2^* + k_3^*)T} \int_0^T C_p^* e^{(k_2^* + k_3^*)t} dt}{\left[\frac{\lambda \cdot V_m^* \cdot K_m}{\phi \cdot V_m \cdot K_m^*} \right] \left[\int_0^T \left(\frac{C_p}{C_p^*} \right) dt - e^{-(k_2^* + k_3^*)T} \int_0^T \left(\frac{C_p}{C_p^*} \right) e^{(k_2^* + k_3^*)t} dt \right]}$$

where R_i is glucose utilization per unit mass of tissue; C_p and C_p^* are the arterial plasma concentrations of glucose and deoxyglucose, respectively; T , the time of decapitation after the infusion of deoxyglucose, was 45 minutes; $C_i^*(T)$ is the amount of radioactivity in the tissue at the end of the experiment; k_1^* , is the rate constant for deoxyglucose entering the tissue, k_2^* and k_3^* are the rate constants for transport of deoxyglucose from the tissue to the plasma and for phosphorylation of deoxyglucose, respectively. λ is the ratio of the distribution volumes of the two hexoses and ϕ equals the fraction of glucose-6-phosphate that enters the glycolytic pathway during

steady state. k_m^* , V_m^* , k_m and V_m are the Michaelis-Menten constants and maximal phosphorylation rates for deoxyglucose and glucose, respectively. The values used for the LC and the rate constants were those determined by Sokoloff et al.(1977) unless otherwise stated.

Lumped constant.

The lumped constant was calculated in SAH animals using the following equation:

$$LC = \frac{k_3^D}{k_3^G} \left[\frac{V_e^M(\infty) C_a}{Me} \right]$$

k_3^D and k_3^G are the rate constants for phosphorylation of deoxyglucose and glucose, respectively. $V_e^M(\infty)$ is the virtual steady state distribution volume for 3-O-methylglucose, C_a is the arterial plasma concentration of glucose and Me is the brain glucose content.

Statistics.

The data were analyzed using the student t-test or the Bonferroni t-test as indicated.

RESULTS AND DISCUSSION.

Paper I.

In this communication, a rat SAH model was presented. The effect of cisternal blood injection was evaluated with bilateral vertebral-basilar angiography at pre-selected time points following the SAH. There was a biphasic vasospasm with a maximal acute spasm at ten minutes and a maximal late spasm at two days post SAH. The spasm was generally diffuse with a duration of two to three days. Increasing the amount of injected blood from 0.07 to 0.3 ml produced a small increase in the degree of spasm. There was a 11.5% mortality associated with cisternal blood injection and angiography. The

SAH animals were drowsy and lethargic during the time period when the angiographical spasm was present, but no focal neurological deficits were noted.

The data demonstrate that it is possible to induce angiographical spasm in the rat with cisternal injection of blood and that there is a good reproducibility in the degree of spasm. Our findings of an increase in the amount of spasm with an increase in the amount of subarachnoid blood is in accordance with findings from a number of clinical (Fisher et al. 1980; Mizukami et al. 1980a) and experimental studies (Espinosa et al. 1982). The spasm duration was shorter in the rat than in humans and other species which might reflect differences in the clearance of blood from the subarachnoid space (Kassell et al. 1985).

Fluorescence microscopy of the major cerebral arteries showed a decrease in fluorescence intensity and a reduction in the number of histochemically visible nerve terminals during the late phase of spasm. The data are in agreement with findings in SAH models in other species (Fraser et al. 1970; Svendgaard et al. 1977; Lobato et al. 1980a). They reflect a depletion in adrenergic transmitter content in the sympathetic perivascular nerves, which could represent an epi-phenomenon or be involved in the development of spasm. A later study has shown that sympathectomy does not prevent the development of spasm (Svendgaard et al. 1985).

Paper II.

A double isotope autoradiographic technique using ^{14}C -iodo-antipyrine and ^3H -deoxyglucose for the simultaneous measurement of CBF and CMRglu was evaluated and applied to the rat SAH model. It was demonstrated first that CBF and CMRglu values were similar in normal animals evaluated with either single isotope or double isotope studies. Two days following a SAH, the timepoint for maximal late spasm, there was a rather uniform 20% reduction in cerebral blood flow together with an increase in glucose utilization of 30%. One-third of the animals also demonstrated focal areas with flow levels of 30% of control values and a deoxyglucose uptake that increased to about 250% of control.

Since the lumped constant may be different post SAH from the value calculated by Sokoloff et al. (1977) for normal rats and thereby

change the CMRglu values, we evaluated the lumped constant in SAH animals in a separate experiment using two glucose analogs ^{14}C deoxyglucose and ^3H -3-O methylglucose. The lumped constant was unchanged in the brain areas outside the foci. In the foci, it increased to 0.61 ± 0.03 (mean $\pm$ S.D) from the normal value 0.48 ± 0.07 (mean $\pm$ S.D.) reflecting the predicted effect of oligoemia.

The global and focal reductions in CBF are in accordance with results from clinical studies in SAH patients using conventional or tomographic CBF measurements (Heilbrun et al. 1972; Gelmers et al. 1979; Ishii 1979; Mickey et al. 1984). Also, the increase in deoxyglucose uptake probably representing an anaerobic glycolysis agrees with the decrease in CMRO_2 described in SAH patients by Kutsuzawa et al. (1968), Grubb et al. (1977), Martin et al. (1984) and Voldby et al. (1985a). The global changes correlate poorly with angiographical vasospasm and are probably a consequent of the SAH per se. It is unlikely that the global changes are produced by an increase in intracranial pressure since they occurred in animals with probably no or minimal changes in intracranial pressure. The focal changes could develop due to the effect of spasm superimposed on the global flow reduction. They develop in boundary areas where the angioarchitectural characteristics are such that vasospasm superimposed on a pre-existing global reduction could result in focal changes. The focal changes could represent pre-infarcts and are probably comparable to the lucent areas noted with CT in SAH patients with vasospasm (Bryan et al. 1979; Saito et al. 1979).

Our data show that there is a "resetting" of the relationship between global flow and metabolism, with a shift to an anaerobic glycolysis. A flow reduction of 20% would normally not be accompanied by an anaerobic glycolysis. Eklöf and Siesjö (1972) and Bruce et al. (1972) demonstrated that reduced cerebral blood flow per se, does not induce anaerobic glycolysis until after a reduction of 50% occurs. It is probable that the presence of blood in the subarachnoid space causes a decrease in the oxygen extraction rate which usually increases as a compensatory mechanism when CBF is reduced. There is in fact both experimental (Fein 1975) and clinical evidence (Voldby et al. 1985a) showing that a decrease in oxygen extraction rate occurs post SAH. How this decrease is produced is unknown. Blood could directly induce the decrease by a toxic effect. However, in view of the uniform nature of the global flow and metabolic changes, it is more likely that a neural mechanism is underlying these changes.

Paper III.

The effect of selective chemical stereotaxic lesions in the lower brain stem on the development of spasm in the rat was presented. A SAH was induced as described in paper I and evaluated with vertebro-basilar angiography. Bilateral lesions of the ascending catecholamine (CA) pathways in the mesencephalon or in the medulla oblongata prior to the SAH prevented the development of both acute and late spasm. The lesions per se did not change the mean vessel diametre of the vertebro-basilar system.

The lesions were produced by 6-hydroxydopamine (6-OHDA) which selectively destroys CA axons and terminals (Tranzer and Thoenen 1967; Ungerstedt 1971). The mesencephalic lesions destroyed ascending fibres from A_6 or the locus coeruleus, A_1 , and A_2 (nomenclature according to Dahlström and Fuxe 1964) while the medullary lesions only destroyed ascending fibres from A_1 and A_2 . A_6 innervates the cortex, hippocampus and to a minor extent the hypothalamus (Amaral and Sinnamon 1977) while A_1 and A_2 almost exclusively innervate the diencephalon (Lindvall and Björklund 1983). Accordingly, animals with bilateral mesencephalic lesions have a NA depletion of between 89 and 100% in the frontal cortex (Norberg et al. 1978; Westerberg et al. 1984). In the present study, there was a NA depletion of $97.0 \pm 1.7\%$ (mean $\pm$ S.E.M.) and $87.3 \pm 6.4\%$ (mean $\pm$ S.E.M.) in the frontal cortex and the diencephalon respectively, confirming the adequacy of the lesion. The animals with medullary lesions had no change in the NA levels in the frontal cortex, but a $65.9 \pm 5.1\%$ (mean $\pm$ S.E.M.) reduction in the diencephalon. This lesion has not been characterized before.

The data indicate that ascending CA projections from A_1 and/or A_2 are involved in the development of spasm. Despite the presence of blood in the subarachnoid space, spasm does not develop. This speaks against the widely held assumption that spasm is produced directly by the vasoconstrictive effect of a factor contained in blood. The data suggests instead that a neural or neurohumoral mechanism is responsible for the development of spasm.

Blood in the subarachnoid space could stimulate sensory nerves on cerebral arteries which could in turn relay "information" to the nucleus tractus solitarius. The nucleus tractus solitarius which is the main center for afferent visceral information (Palkovits and Zaborisky 1977; Sawchenko and Swanson 1981) is interconnected with

A₁ and A₂ (Loewy et al. 1981). From A₁ and/or A₂ information might be transmitted to effector neurons in other brain regions. A₁ and A₂ project to the diencephalon and in particular to the hypothalamus-pituitary (Palkovits et al. 1980; Sawchenko and Swanson 1981). The hypothalamus-pituitary could be involved in the development of spasm via an autonomic mechanism. However, it is not likely that an autonomic mechanism mediated either via the adrenal medulla or superior cervical ganglia is involved. We have shown that rats post adrenal demedullation or cervical ganglionectomy still develop spasm (Svendgaard et al. 1985). Instead, the hypothalamus and/or pituitary could be involved via a humoral or intrinsic neural mechanism.

Paper IV.

Local CBF and CMRglu were evaluated two days post SAH using the double isotope autoradiographic technique in rats with lower brainstem lesions. It was found that lesioning in the mesencephalon of the ascending CA pathways from A₆, A₁ and A₂ or lesioning in the medulla oblongata of the ascending fibres from A₁ and A₂ prevented the development of the global and focal CBF and CMRglu changes seen in normal animals post SAH. The data indicate that A₁ and/or A₂ are essential for the development of the post SAH flow and metabolic changes.

The lesions per se did not change the baseline flow and metabolism as compared to sham lesioned animals. Previous studies measuring CBF in rats with mesencephalic or locus coeruleus lesions using a tissue uptake technique (Norberg et al. 1978), the ¹³³-xenon wash-out method (Dahlgren et al. 1981) or an autoradiographic technique (Blomqvist et al. 1984) also found no significant change in CBF. Local CMRglu has been evaluated using an autoradiographic technique (Schwartz 1978; Savaki et al. 1984) in rats with locus coeruleus lesions. No major influence of the locus coeruleus on the metabolism was found. CBF or CMRglu after selective lesions of the ascending fibres from A₁ and A₂ has not been investigated previously.

The lesions were produced by 6-OHDA and controlled with chemical analysis of the NA content in the frontal cortex. The results were in agreement with data presented in paper III.

A₁ and A₂ are known to project to a number of brain regions

which may influence flow or metabolism such as the parabrachial complex (Ricardo and Koh 1978, Loewy et al. 1981) the raphe nuclei (Levitt and Moore 1979) and the hypothalamus (Ricardo and Koh 1978; Lindvall and Björklund 1983). It is unlikely that serotonergic projections from the raphe nuclei are involved. We have found that animals with chemical lesions of the serotonergic systems subjected to a SAH still develop changes in CBF and CMRglu (unpublished observation). The hypothalamus is the most likely effector organ either via direct projections from A_1 and/or A_2 or indirectly via the parabrachial complex. The presence of blood in the subarachnoid space seems to trigger via a neural mechanism not only vasospasm, but also the CBF reduction and anaerobic glycolysis seen post SAH. Different blood components appearing at different time points after the SAH could trigger via the sensory nerves, the relay of "information" to neuronal subgroups within the nucleus tractus solitarius or A_2 that project to effector target neurons producing a variability in the response concerning spasm, flow and metabolism.

Paper V.

The role of the hypothalamus-pituitary in the development of spasm was evaluated by applying selective lesions within this region to the rat model. The lesions per se did not change mean vessel diameter in the vertebro-basilar system.

It was shown that intravenous injection of 6-OHDA prevents the development of vasospasm post SAH. Intravenous 6-OHDA destroys CA containing terminals in both the sympathetic system (Malmfors 1971), and in cerebral regions not protected by the blood brain barrier such as the median eminence, posterior and intermediate lobe of the pituitary, subfornical organ and area postrema (Baumgarten et al. 1972; Jonsson et al. 1972; Brizzee et al. 1978; Palazzo et al. 1978; Day and Willoughby 1980; Smith et al. 1982; Lim et al. 1984). The absence of spasm in the 6-OHDA treated animals is unlikely to be due to damage of the sympathetic system only, since animals with cranial sympathectomy prior to a SAH, still develop spasm (Svendgaard et al. 1985). It is more likely that the effect is due to a central action of the drug.

In view of our earlier findings that lesioning of the ascending CA pathways from A_1 and A_2 to the hypothalamus-pituitary pre-

vented spasm, it was most probable that the median eminence and/or the neurointermediate lobe were the regions involved. Caudal pituitary stalk lesion did not affect the degree of spasm, while no spasm developed in animals with lesion of the median eminence indicating that the median eminence is the site co-responsible with A₁ and/or A₂ for spasm. Further, given the resistance of the DA terminals in the median eminence to the effect of 6-OHDA (Jonsson et al. 1972; Cuello et al. 1974), it is most likely that it is the NA terminals in the median eminence which are involved in spasm. Supportive evidence is provided by the development of spasm post SAH in animals three weeks after the 6-OHDA treatment (unpublished observations); the time point when the NA terminals reappear in the median eminence (Smith et al. 1982).

The median eminence could produce vasoconstriction in the cerebral arteries via a humoral or neural mechanism. It is known that NA neurons terminating in the median eminence can facilitate the release of neuropeptides (Fuxe et al. 1976; Sarkar et al. 1981). The neuropeptides may be liberated into cerebrospinal fluid (Knigge et al. 1976; Bergland and Page 1978). We have examined cerebrospinal fluid from both patients and rats post SAH and found a hitherto unknown spasmogenic compound which is probably a peptide (to be published). The substance is absent in cerebrospinal fluid from SAH rats with median eminence or lower brain stem lesions (to be published). However, it is also known that there are multiple non-adrenergic neural connections (Bujs et al. 1978; Recht et al. 1982) from hypothalamic nuclei interconnected with the median eminence (Palkovits 1982) that could potentially produce neurogenic vasoconstriction. It remains to be determined which type of end-stage mechanism, humoral or neural, underlies spasm.

CONCLUSIONS

1. An experimental SAH in the rat induces a reproducible angiographically demonstrable vasospasm that has a biphasic time course.
2. During the late phase of vasospasm, the animals showed a global reduction in CBF accompanied by an increase in deoxyglucose uptake consistent with an anaerobic glycolysis. In addition, about one-

third of the animals had focal low flow areas with correspondingly high deoxyglucose accumulation.

3. Neurons in the A_1 and A_2 nuclei projecting in the central tegmental tract to the hypothalamus are essential for the development of both the acute and late vasospasm and the flow and metabolic changes seen during the late spasm phase.

4. The median eminence is a site in the hypothalamus coresponsible for the development of vasospasm.

5. Subarachnoid blood does not induce vasospasm by a direct vascular effect, but via a neural mechanism.

ACKNOWLEDGEMENTS

The studies were carried out at the Neurosurgical Research Department, University of Lund, Lund, Sweden.

I wish to express my profound gratitude to my tutor, Associate Professor Niels Aage Svendgaard, who introduced me to the subject of cerebral vasospasm. His knowledge has challenged and inspired me, and his support and guidance have proven invaluable.

I also thank:

Lektor Nils Henrik Diemer for help with the analysis of the autoradiograms and valuable discussion of the data.

Professor Carl-Axel Thulin for encouraging me to enter the field of research.

Assistant Professor Olle Lindvall for valuable discussions of anatomy.

Associate Professor Claus Rerup for valuable discussions of statistics.

Anna Atanasiadu for excellent laboratory assistance.

Eevi Paasonen for typing the manuscript.

Ulla-Britt Andersson, Anitha Bruun, Yvette Jönsson, Ann-Marie Olsson, Kristina Strimell and Kerstin Stureson for skillful technical assistance.

The studies were supported by grants from:

Einar Björkelund Foundation

Herman Gross Foundation

Anna-Lisa and Sven-Eric Lundgren Foundation

Elsa Schmitz Foundation

Thorsten and Elsa Segerfalk Foundation

Danish Medical Research Council

Medical Faculty, University of Lund

Swedish Medical Association

Swedish Medical Research Council

REFERENCES

- Alksne JF (1974) Myonecrosis in chronic experimental vasospasm. *Surgery* 76:1-7
- Alksne JF, Greenhoot JH (1974) Experimental catecholamine-induced chronic cerebral vasospasm. Myonecrosis in vessel wall. *J Neurosurg* 41:440-445
- Allcock JM (1966) Arterial spasm in subarachnoid haemorrhage: A clinical and experimental study. *Acta Radiol* 5:73-83
- Allcock JM, Drake CG (1965) Ruptured intracranial aneurysms: the role of arterial spasm. *J Neurosurg* 22:21-29
- Allen GS, Ahn HS, Preziosi TJ, Battye R, Boone SC, Chou SN, Kelly DL, Weir BK, Crabbe RA, Lavik PJ, Rosenbloom SB, Dorsey FC, Ingram CR, Mellits DE, Bertsch LA, Boisvert DPJ, Hundley MB, Johnson RK, Strom JA, Transou CR (1983) Cerebral arterial spasm – a controlled trial of nimodipine in patients with subarachnoid hemorrhage. *N Engl J Med* 308:619-624
- Allen GS, Bahr AL (1979) Cerebral arterial spasm: Part 10. Reversal of acute and chronic spasm in dogs with orally administered nifedipine. *Neurosurgery* 4:43-47
- Allen GS, Gold LHA, Chou SN, French LA (1974a) Cerebral arterial spasm. Part 3: In vivo intracisternal production of spasm by serotonin and blood and its reversal by phenoxybenzamine. *J Neurosurg* 40:451-458
- Allen GS, Henderson LM, Chou SN, French LA (1974b) Cerebral arterial spasm. Part 2: In vitro contractile activity of serotonin in human serum and CSF on the canine basilar artery and its blockage by methysergide and phenoxybenzamine. *J Neurosurg* 40:442-450
- Allen GS, Henderson LM, Chou SN, French LA (1974c) Cerebral arterial spasm. Part 1: In vitro contractile activity of vasoactive agents on canine basilar and middle cerebral arteries. *J Neurosurg* 40:433-441
- Amaral DG, Sinnamon HM (1977) The locus coeruleus: neurobiology of a central noradrenergic nucleus. *Progress in Neurobiology* 9:147-196
- Armstrong FS, Hayes GJ (1961) Segmental cerebral arterial constriction associated with pheochromocytoma. Report of a case with arteriograms. *J Neurosurg* 18:843-846
- Auer LM (1984) Acute operation and preventive nimodipine improve outcome in patients with ruptured cerebral aneurysms. *Neurosurgery* 15: 57-66
- Barry KJ, Gogjian MA, Stein BM (1979) Small animal model for investigation of subarachnoid hemorrhage and cerebral vasospasm. *Stroke* 10:538-541
- Barry KJ, Mikkelsen RB, Shucart W, Keough EM, Garvis V (1985) Isolation and characterization of calcium-accumulating sub-cellular membrane fractions from cerebral arteries. *J. Neurosurg* 62:729-736
- Baumgarten HG, Björklund A, Holstein AF, Nobin A (1972) Organization and ultrastructural identification of the catecholamine nerve terminals in the neural lobe and pars intermedia of the rat pituitary. *Z Zellforsch* 126:483-517
- Bergland RM, Page RB (1978) Can the pituitary secrete directly to the brain? (Affirmative anatomical evidence). *Endocrinology* 102:1325-1338
- Bergvall U, Galera R (1969) Time relationship between subarachnoid haemorrhage, arterial spasm, changes in cerebral circulation and posthaemorrhagic hydrocephalus. *Acta Radiol (Diagn) (Stockh)* 9:229-237

- Bergvall U, Steiner L, Forster DMC (1973) Early pattern of cerebral circulatory disturbances following subarachnoid haemorrhage. *Neuroradiology* 5:24-32
- Björklund A, Falck B, Owman Ch (1972) Fluorescence microscopic and micro-spectrofluorometric techniques for the cellular localization and characterization of biogenic amines. In *The Thyroid and Biogenic Amines, Methods of Investigative and Diagnostic Endocrinology, Vol 1.* (Rall JE, Kapin IJ, eds) Amsterdam, North Holland, pp 318-368
- Blaumanis OR, Grady PA (1982) Experimental cerebral vasospasm: resolution by chlorpromazine. *Surg Neurol* 17:263-268
- Blomqvist P, Lindvall O, Wieloch T (1984) Delayed postischemic hypoperfusion: Evidence against involvement of the noradrenergic locus ceruleus system. *J Cereb Blood Flow Metabol* 4:425-429
- Boisvert DPJ, Pickard JD, Graham DI, Fitch W (1979) Delayed effects of subarachnoid haemorrhage on cerebral metabolism and the cerebrovascular response to hypercapnia in the primate. *J Neurol Neurosurg Psychiatry* 42:892-898
- Boullin DJ, Brandt L, Ljunggren B, Tagari P (1981) Vasoconstrictor activity in cerebrospinal fluid from patients subjected to early surgery for ruptured intracranial aneurysms. *J Neurosurg* 55:237-245
- Boullin DJ, Mohan J, Grahame-Smith DG (1976) Evidence for the presence of a vasoactive substance (possibly involved in the aetiology of cerebral arterial spasm) in cerebrospinal fluid from patients with subarachnoid haemorrhage. *J Neurol Neurosurg Psychiatry* 39:756-766
- Boullin DJ, Bunting S, Blaso WP, Hunt TM, Moncada S (1979) Responses of human and baboon arteries to prostaglandin endoperoxides and biologically generated and synthetic prostacyclin: their relevance to cerebral arterial spasm in man. *Br J Clin Pharmacol* 7:139-147
- Brandt L, Andersson KE, Bengtsson B, Edvinsson L, Ljunggren B, Mackenzie ET (1979) Effects of nifedipine on pial arteriolar calibre: an in vivo study. *Surg Neurol* 12:349-352
- Brandt L, Ljunggren B, Andersson KE, Hindfelt B, Teasdale G (1981) Vasoconstrictive effects of human post-hemorrhagic cerebrospinal fluid on cat pial arterioles in situ. *J Neurosurg* 54:351-356
- Brawley BW, Strandness DE Jr, Kelly WA (1968) The biphasic response of cerebral vasospasm in experimental subarachnoid hemorrhage. *J Neurosurg* 28:1-8
- Brizzee KR, Palazzo MC, Klara PM, Hofer H (1978) Effects of systemic administration of 6-hydroxydopamine on the circumventricular organs in nonhuman primates. I. Area postrema. *Cell Tiss Res* 187:115-127
- Bruce DA, Schultz H, Vapalahti M, Gunby N, Langfitt TW (1972) Interactions between cerebral blood flow and cerebral metabolism. *Surg Forum* 23:417-419
- Bryan RN, Shah CP, Hilal S (1979) Evaluation of subarachnoid hemorrhage and cerebral vasospasm by computed tomography. *CT* 3:144-152
- Bujs RM, Swaab DF, Dogterom J, van Leeuwen FW (1978) Intra- and extrahypothalamic vasopressin and oxytocin pathways in the rat. *Cell Tissue Res* 186:423-433
- Chan RC, Durity FA, Thompson GB, Nugent RA, Kendall M (1984) The role of the prostacyclin-thromboxane system in cerebral vasospasm following induced subarachnoid hemorrhage in the rabbit. *J Neurosurg* 61: 1120-1128

- Chow RWB, Newton TH, Smith MC, Adams JE (1968) Cerebral vasospasm induced by subarachnoid blood and serotonin: an angiographical study. *Invest Radiol* 3:402-407
- Chyatte D, Rusch N, Sundt TM Jr (1983) Prevention of chronic experimental cerebral vasospasm with ibuprofen and high-dose methylprednisolone. *J Neurosurg* 59:925-932
- Clower BR, Haining JL, Smith RR (1980) Pathophysiological changes in the cerebral artery after subarachnoid hemorrhage. In *Cerebral Arterial Spasm* (Wilkins RH, ed) Baltimore/London, Williams & Wilkins, pp 124-131
- Conway LW, McDonald LW (1972) Structural changes of the intradural arteries following subarachnoid hemorrhage. *J Neurosurg* 37:715-723
- Crompton MR (1964) The pathogenesis of cerebral infarction following the rupture of cerebral berry aneurysms. *Brain* 87:491-510.
- Cuello AC, Shoemaker WJ, Ganong WF (1974) Effect of 6-hydroxydopamine on hypothalamic norepinephrine and dopamine content, ultrastructure of the median eminence, and plasma corticosterone. *Brain Res* 78:57-69
- Dahlgren N, Lindvall O, Sakabe T, Stenevi U, Siesjö BK (1981) Cerebral blood flow and oxygen consumption in the rat brain after lesions of the noradrenergic locus coeruleus system. *Brain Res* 209:11-23
- Dahlström A, Fuxe K (1964) Evidence for the existence of monoamine-containing neurons in the central system. I. Demonstration of monoamines in the cell bodies of brain stem neurons. *Acta Physiol Scand* 62 (Suppl 232):1-55
- Dale J, Landmark KH, Myhre E (1983) The effects of nifedipine, a calcium antagonist, on platelet function. *Am Heart J* 105:103-105
- Day TA, Willoughby JO (1980) Noradrenergic afferents to median eminence: inhibitory role in rhythmic growth hormone secretion. *Brain Res* 202:335-345
- Diemer NH, Rosenørn J (1981) Determination of local cerebral blood flow and glucose metabolism or transfer by means of a double autoradiographic method. *J Cereb Blood Flow Metabol* 1:572-573
- DuBoulay G (1963) Distribution of spasm in the intracranial arteries after subarachnoid haemorrhage. *Acta Radiol (Diagn)* (Stockh), 1:257-266
- Echlin FA (1965) Spasm of basilar and vertebral arteries caused by experimental subarachnoid hemorrhage. *J Neurosurg* 23:1-11
- Ecker A, Riemenschneider PA (1951) Arteriographic demonstration of spasm of the intracranial arteries: with special reference to saccular arterial aneurisms. *J Neurosurg* 8:660-667
- Eklöf B, Siesjö BK (1972) The effect of bilateral carotid artery ligation upon the blood flow and the energy state of the rat brain. *Acta Physiol Scand* 86:155-165
- Eldevik OP, Kristiansen K, Torvik A (1981) Subarachnoid hemorrhage and cerebrovascular spasm. Morphological study of intracranial arteries based on animal experiments and human autopsies. *J Neurosurg* 55:869-876
- Ellis F, Nies AS, Oates JA (1977) Cerebral arterial smooth muscle contraction by thromboxane A₂. *Stroke* 8:480-483
- Endo S, Suzuki J (1977) Experimental cerebral vasospasm after subarachnoid hemorrhage. Development and degree of vasospasm. *Stroke* 8:702-707
- Eriksson B-M, Persson B-A (1982) Determination of catecholamines in rat heart

- tissue and plasma samples by liquid chromatography with electrochemical detection. *J Chromatogr* 228:143-154
- Espinosa F, Weir B, Boisvert D, Overton T, Castor W (1982) Chronic cerebral vasospasm after large subarachnoid hemorrhage in monkeys. *J Neurosurg* 57:224-232
- Espinosa F, Weir B, Overton T, Castor W, Grace M, Boisvert D (1984a) A randomized placebo-controlled double-blind trial of nimodipine after SAH in monkeys. Part 1: Clinical and radiological findings. *J Neurosurg* 60:1167-1175
- Espinosa F, Weir B, Shnitka T, Overton T, Boisvert D (1984b) A randomized placebo-controlled double-blind trial of nimodipine after SAH in monkeys. Part 2: Pathological findings. *J Neurosurg* 60:1176-1185
- Falck B, Hillarp NA, Thieme G, Torp A (1962) Fluorescence of catecholamines and related compounds condensed with formaldehyde. *J Histochem Cytochem* 10:348-354
- Farrar JK, Gamache FW, Ferguson GG, Baker J, Varkey GP, Drake CG (1981) Effects of profound hypotension on cerebral blood flow during surgery for intracranial aneurysms. *J Neurosurg* 55:857-864
- Fein JM (1975) Cerebral energy metabolism after subarachnoid hemorrhage. *Stroke* 6:1-8
- Fein JM, Flor WJ, Cohan SL, Parkhurst J (1974) Sequential changes of vascular ultrastructure in experimental cerebral vasospasm: Myonecrosis of subarachnoid arteries. *J Neurosurg* 41:49-58
- Ferguson GC, Harper AM, Fitch W, Rowan JO, Jennett B (1972) Cerebral blood flow measurements after spontaneous subarachnoid haemorrhage. *Eur Neurol* 8:15-22
- Fisher CM, Kistler JP, Davis JM (1980) Relation of cerebral vasospasm to subarachnoid hemorrhage visualized by computerized tomographic scanning. *Neurosurgery* 6:1-9
- Flamm ES, Kim J, Lin J, Ransohoff J (1975) Phosphodiesterase inhibitors and cerebral vasospasm. *Arch Neurol* 32:569-571
- Fletcher TM, Taveras JM, Pool JLC (1959) Cerebral vasospasm in angiography for intracranial aneurysms: Incidence and significance in one hundred consecutive angiograms. *Arch Neurol* 1:38-47
- Forster C, Mohan J, Whalley ET (1980) Interaction of fibrin degradation products and 5-hydroxytryptamine on various vascular smooth muscle preparations: possible role in cerebral vasospasm. In *Cerebral Arterial Spasm* (Wilkins RH, ed) Baltimore/London, Williams & Wilkins, pp 186-189
- Fox JL, Yasargil MG (1975) The relief of intracranial vasospasm: An experimental study with methylprednisolone and cortisol. *Surg Neurol* 3:214-218
- Fraser RAR, Stein BM, Barrett RE, Pool JL (1970) Noradrenergic mediation of experimental cerebrovascular spasm. *Stroke* 1:356-362
- Fukumori T, Tani E, Maeda Y (1984) Effect of selective inhibitor of thromboxane A_2 synthetase on experimental cerebral vasospasm. *J Neurosurg* 59:829-834
- Fukumori T, Tani E, Maeda Y, Sukenaga A (1983) Effects of prostacyclin and indomethacin on experimental delayed cerebral vasospasm. *J Neurosurg* 59:829-834

- Fuxe K, Hökfelt T, Agnati L, Löfström A, Everitt BJ, Johansson O, Jonsson G, Wuttke W, Goldstein M (1976) Role of monoamines in the control of gonadotropin secretion. In *Neuroendocrine Regulation of Fertility* (Kumar A, ed) Basel, Karger, pp 124-140
- Gabrielsen TO, Greitz T (1970) Normal size of the internal carotid, middle cerebral and anterior cerebral arteries. *Acta Radiol (Diagn)* (Stockh) 10:1-10
- Gavras H, Andrews P, Papadakis N (1981) Reversal of experimental delayed cerebral vasospasm by angiotensin-converting enzyme inhibition. *J Neurosurg* 55:884-888
- Gelmers HJ, Beks JWF, Journée HL (1979) Regional cerebral blood flow in patients with subarachnoid haemorrhage. *Acta Neurochir* 47:245-251
- Gjedde A, Diemer NH (1985) Double-tracer study of the fine regional blood-brain glucose transfer in the rat by computer-assisted autoradiography. *J Cereb Blood Flow Metabol* 5:282-289
- Gjedde A, Hansen AJ, Siemkowicz E (1980) Rapid simultaneous determination of regional blood flow and blood-brain glucose transfer in brain of rat. *Acta Physiol Scand* 108:321-330
- Graf CJ, Nibbelink DW (1974) Cooperative study of intracranial aneurysms and subarachnoid haemorrhage: report on a randomized treatment study. III. Intracranial surgery. *Stroke* 5:559-601
- Graham DI, MacPherson P, Pitts LH (1983) Correlation between angiographic vasospasm, hematoma, and ischemic brain damage following SAH. *J Neurosurg* 59:223-230
- Greitz T (1964) Angiography in tuberculous meningitis. *Acta Radiol (Diagn)* (Stockh) 2:369-378
- Griffith HB, Cummins BH, Thomson JLG (1972) Cerebral arterial spasm and hydrocephalus in leaking arterial aneurysms. *Neuroradiology* 4: 212-214
- Grotenhuis JA, Bettag W, Fiebach BJO, Dabir K (1984) Intracarotid slow bolus injection of nimodipine during angiography for treatment of cerebral vasospasm after SAH. *J Neurosurg* 61:231-240
- Grubb RL, Raichle ME, Eichling JO, Gado MH (1977) Effects of subarachnoid hemorrhage on cerebral blood volume, blood flow, and oxygen utilization in humans. *J Neurosurg* 46:446-453
- Handa H, Nagasawa S, Yonekawa Y, Narvo Y, Oda Y (1983) New treatment of cerebral vasospasm with fluosol-DA 20%. Protective effect on cerebral ischemia and change of cerebral blood flow (CBF). *Prog Cir Biol Res* 122:299-306
- Hashi K, Matsuoka Y, Tanaka K, Ohkawa N (1980) Treatment of cerebral vasospasm with large doses of hydrocortisone. In *Cerebral Arterial Spasm* (Wilkins RH, ed) Baltimore/London, Williams & Wilkins, pp 611-618
- Hashi K, Meyer JS, Shinmaru S, Welch KMA, Teraura T (1972) Cerebral hemodynamic and metabolic changes after experimental subarachnoid hemorrhage. *J Neurol Sci* 17:1-14
- Hass W (1981) Beyond cerebral blood flow, metabolism and ischemic thresholds. An examination of the role of calcium in the initiation of cerebral infarction. In *Cerebral Vascular Disease* (Meyer JS, Lechner H, Reidich M, Ott EO, Aranibar A, eds) Amsterdam, Excerpta Medica, pp 3-17

- Hayashi S, Toda N (1977) Inhibition by Ca^{2+} , verapamil and papaverine of Ca^{2+} -induced contractions in isolated cerebral and peripheral arteries of the dog. *Brit J Pharmacol* 60:35-43
- Heilbrun MP, Olesen J, Lassen NA (1972) Regional cerebral blood flow studies in subarachnoid hemorrhage. *J Neurosurg* 37:36-44
- Heyman A, Saltzman HA, Whalen RE (1966) The use of hyperbaric oxygenation in the treatment of cerebral ischemia and infarction. *Circ* 33/34 (suppl) 20-27
- Hughes JJ, Schianchi PM (1978) Cerebral artery spasm. A histological study at necropsy of the blood vessels in cases of subarachnoid hemorrhage. *J Neurosurg* 48:515-525
- Hunt TM, DuBoulay GH, Blaso WP, Forster DMC, Boullin DJ (1979) Relationship between presence of vasoconstrictor activity in cerebrospinal fluid and time after subarachnoid haemorrhage from rupture of cerebral arterial aneurysms. *J Neurol Neurosurg Psychiatry* 42:625-634
- Ishii R (1979) Regional cerebral blood flow in patients with ruptured intracranial aneurysms. *J Neurosurg* 50:587-594
- Jakubowski J, Bell BA, Symon L, Zawirski MB, Francis DM (1982) A primate model of subarachnoid hemorrhage: change in regional cerebral blood flow, autoregulation, carbon dioxide reactivity, and central conduction time. *Stroke* 13:601-611
- James IM (1968) Changes in cerebral blood flow and in systemic arterial pressure following spontaneous subarachnoid hemorrhage. *Clin Sci* 35:11-22
- Jonsson G, Fuxe K, Hökfelt T (1972) On the catecholamine innervation of the hypothalamus, with special reference to the median eminence. *Brain Res* 40:271-281
- Kapp J, Mahaley MS, Odom GL (1968) Cerebral arterial spasm. Part 2: Experimental evaluation of mechanical and humoral factors in pathogenesis. *J Neurosurg* 29:339-349
- Kassell NF, Peerless SJ, Durward QJ (1982) Treatment of ischemic deficits from vasospasm with intravascular volume expansion and induced arterial hypertension. *Neurosurgery* 11:337-343
- Kassell NF, Sasaki T, Colohan ART, Nazar G (1985) Cerebral vasospasm following aneurysmal subarachnoid hemorrhage. *Stroke* 16:562-572
- Kassell NF, Törner JC, Adams HP (1984) Antifibrinolytic therapy in the acute period following aneurysmal subarachnoid hemorrhage: preliminary observations from the Cooperative Aneurysm Study. *J Neurosurg* 61:225-230
- Kato Y, Sano H, Katada K, Kanno T (1985) Experimental and clinical study in the use of intrathecal alpha-tocopherol in vasospasm. In *Timing of Aneurysm Surgery* (Auer LM, ed) Berlin/New York, Walter de Gruyter, pp 615-626
- Kazda SR, Towart R (1982) Nimodipine: a new calcium antagonistic drug with a preferential cerebrovascular action. *Acta Neurochir (Wien)* 63:259-265
- Kelly PJ, Gorten RJ, Grossman RG, Eisenberg HM (1977) Cerebral perfusion, vascular spasm, and outcome in patients with ruptured intracranial aneurysms. *J Neurosurg* 47:44-49
- Kindt GW, McGillicuddy J, Pritz M, Giannotta S (1980) Hypertension and hypervolemia as therapy for patients with vasospasm. In *Cerebral Arterial Spasm*

- (Wilkins RH, ed) Baltimore/London, Williams & Wilkins, pp 659-664
- Knigge KM, Joseph SA, Sladek JR, Notter MF, Morris M, Sundberg DK, Holzwarth MA, Hoffman GE, O'Brien L (1976) Uptake and transport activity of the median eminence of the hypothalamus. *Int Rev Cytol* 45:383-408
- Kutsusawa T, Takahashi S, Saito C, Takahashi S, Sato T, Iwabuchi T (1968) Studies of cerebral hemodynamics in subarachnoid hemorrhage. *Tohoku J Exp Med* 94:407-415
- Kuwayama A, Zervas NT, Belson R, Shintani A, Pickren K (1972) A model for experimental cerebral arterial spasm. *Stroke* 3:49-56
- Kågstöm E, Greitz T, Hanson J, Galera R (1966) Changes in cerebral blood flow after subarachnoid haemorrhage. *Excerpta Med Int Cong Series* 110:629-633
- Kågstöm E, Nilsson PE, Svendgaard NA (1969) Clinical and experimental spasm of the cerebral vessels. *Excerpta Med Int Congr Series* 193:60
- Kågstöm E, Palma L (1972) Influence of antifibrinolytic treatment on the morbidity in patients with subarachnoid hemorrhage. *Acta Neurol Scand* 48:257
- Landau B, Ransohoff J (1968) Prolonged cerebral vasospasm in experimental subarachnoid hemorrhage. *Neurology* 18:1056-1065
- Lee TJ-F, McIlhany MP, Sarwinski S (1984) Erythrocyte extracts enhance neurogenic vasoconstriction of dog cerebral arteries in vitro. *J Cereb Blood Flow Metabol* 4:474-476
- Levitt P, Moore RY (1979) Origin and organization of brainstem catecholamine innervation in the rat. *J Comp Neurol* 186:505-528
- Lim AT, Smith GC, Clements JA, Funder JW (1984) Stress, dopaminergic blockade and median eminence – neurointermediate lobe catecholamine depletion: effects on hypothalamic, pituitary and plasma immunoreactive B-endorphin. *Clin Exp Pharmacol Physiol* 11:221-229
- Lindvall O, Björklund A (1983) Dopamine- and norepinephrine-containing neuron systems: their anatomy in the rat brain. In: *Chemical Neuroanatomy* (Emson PC, ed) New York, Raven Press, pp 229-255
- Liszcak TM, Black PMcL, Tzouras A, Foley L, Zervas NT (1984) Morphological changes of the basilar artery, ventricles, and choroid plexus after experimental SAH. *J Neurosurg* 61:486-493
- Little JR (1978) Modification of acute focal ischemia by treatment with mannitol. *Stroke* 9:4-9
- Little JR, Slugg RM, Latchaw JP Jr, Lesser RP (1981) Treatment of acute focal cerebral ischemia with concentrated albumin. *Neurosurgery* 9:552-558
- Ljunggren B, Brandt L, Säveland H, Nilsson PE, Cronqvist S, Andersson KE, Vinge E (1984) Outcome in 60 consecutive patients treated with early aneurysm operation and intravenous nimodipine. *J Neurosurg* 61:864-873
- Lobato RD, Marin J, Salaices M, Burgos J, Rivilla F, Garcia AG (1980a) Effect of experimental subarachnoid hemorrhage on the adrenergic innervation of cerebral arteries. *J Neurosurg* 53:477-479
- Lobato RD, Marin J, Salaices M, Rivilla F, Burgos J (1980b) Cerebrovascular reactivity to noradrenaline and serotonin following experimental subarachnoid hemorrhage. *J Neurosurg* 53:480-485
- Loewy AD, Wallach JH, McKellar S (1981) Efferent connections of the ventral medulla oblongata in the rat. *Brain Res Rev* 3:63-80

- Lyons EL, Leeds NE (1967) The angiographic demonstration of arterial vascular disease in purulent meningitis: Report of a case. *Radiology* 88:935-938
- MacPherson P, Graham DI (1978) Correlation between angiographic findings and the ischaemia of head injury. *J Neurol Neurosurg Psychiatry* 41:122-127
- Malmfors T (1971) The effects of 6-hydroxydopamine on the adrenergic nerves as revealed by the fluorescence histochemical method. In *6-Hydroxydopamine and Catecholamine Neurons* (Malmfors T, Thoenen H, eds) Amsterdam, North-Holland, pp 47-58
- Martin WRW, Baker RP, Grubb RL, Raichle ME (1984) Cerebral blood volume, blood flow, and oxygen metabolism in cerebral ischaemia and subarachnoid haemorrhage: an in vivo-study using positron emission tomography. *Acta Neurochir* 70:3-9
- Martin V, Villani GM, Jothianandan D, Furchgott RF (1985) Selective blockade of endothelium dependent and glycerol-trinitrate induced relaxation by hemoglobin and methylene blue in the rabbit aorta. *J Pharmacol Exp Ther* 232:708-716
- Mayberg MR, Houser OW, Sundt TM (1978) Ultrastructural changes in feline arterial endothelium following subarachnoid hemorrhage. *J Neurosurg* 48:49-57
- McMurty JG, Pool JL, Nova HR (1967) The use of rheomacrodex in the surgery of intracranial aneurysms. *J Neurosurg* 26:218-222
- Mendelow AD, McCalden TA, Hattingh J, Coull A, Rosendorff C, Eidelman BH (1981) Cerebrovascular reactivity and metabolism after subarachnoid hemorrhage in baboons. *Stroke* 12:58-65
- Mickey B, Vorstrup S, Voldby B, Lindewald H, Harmsen A, Lassen NA (1984) Serial measurement of regional cerebral blood flow in patients with SAH using ¹³³Xe inhalation and emission computerized tomography. *J Neurosurg* 60:916-922
- Mizukami M, Kawase T, Tazawa T, Nagata K, Yunoki K, Yoshida Y (1980b) Hypothesis and clinical evidence for the mechanism of chronic cerebral vasospasm after subarachnoid hemorrhage. In *Cerebral Arterial Spasm* (Wilkins RH, ed) Baltimore/London, Williams & Wilkins, pp 97-106
- Mizukami M, Kawase T, Usami T, Tazawa T (1982) Prevention of vasospasm by early operation with removal of subarachnoid blood. *Neurosurgery* 10:301-307
- Mizukami M, Takemae T, Tazawa T, Kawase T, Matsuzaki T (1980a) Value of computerized tomography in the prediction of cerebral vasospasm after aneurysm rupture. *Neurosurgery* 7:583-586
- Nagai H, Noda S, Mabe H (1975) Experimental cerebral vasospasm. Part 2: Effects of vasoactive drugs and sympathectomy on early and late spasm. *J Neurosurg* 42:420-428
- Nagai H, Suzuki Y, Sugiura M, Noda S, Mabe H (1974) Experimental cerebral vasospasm. Part 1: Factors contributing to early spasm. *J Neurosurg* 41:285-292
- Nagasawa S, Handa H, Naruo Y, Moritake K, Hayashi K (1982) Experimental cerebral vasospasm. Arterial wall mechanisms and connective tissue composition. *Stroke* 13:595-600
- Nakano M, Tani E, Fukumori T, Yokota M (1984) Effects of chlorpromazine on experimental delayed cerebral vasospasm. *J Neurosurg* 61:857-863
- Norberg K, Scatton B, Korf J (1978) Amphetamine-induced increase in rat cerebral blood flow: apparent lack of catecholamine involvement. *Brain Res* 149:165-174

- Nosko M, Weir B, Krueger C, Cook D, Norris S, Overton T, Boisvert D (1985) Nimodipine and chronic vasospasm in monkeys. Part 1: Clinical and radiological findings. *Neurosurgery* 16: 129-136
- Oba M, Mizoi K, Fujimoto S, Yashimoto T, Suzuki J (1985) Effect of postischemic administration of mannitol, vitamin E, dexamethasone and perfluorochemicals on cerebral ischemia. An experimental study. In *Cerebral Revascularization for Stroke* (Spetzler RF, Carter LP, Selman WR, Martin NA, eds) New York, Thieme Stratton, pp 267-274
- Ohta T, Kajikawa H, Yoshikawa Y, Shimizu K, Funatsu N, Yamamoto M, Toda N (1980) Cerebral vasospasm and hemoglobins: clinical and experimental studies. In *Cerebral Arterial Spasm* (Wilkins RH, ed) Baltimore/London, Williams & Wilkins, pp 166-172
- Osaka K (1977) Prolonged vasospasm produced by the breakdown products of erythrocytes. *J Neurosurg* 47:403-411
- Palazzo MC, Brizzee KR, Hofer H, Mehler R (1978) Effects of systemic administration of 6-hydroxydopamine on the circumventricular organs in non-human primates. II. Subfornical organ. *Cell Tiss Res* 191:141-150
- Palkovits M (1982) Neuropeptides in the median eminence: Their sources and destinations. *Pepides* 3:299-303
- Palkovits M, Zaborsky L (1977) Neuroanatomy of central cardiovascular control. Nucleus tractus solitarii: afferent and efferent neuronal connections in relation to the baroreceptor reflex arc. *Arc Prog Brain Res* 47:9-34
- Palkovits M, Zaborsky L, Feminger A, Mezey E, Fekete MIK, Herman JP, Kanyicska B, Szabó D (1980) Noradrenergic innervation of the rat hypothalamus: experimental biochemical and electron microscopic studies. *Brain Res* 191:161-171
- Peerless SJ (1980) Postoperative cerebral vasospasm without subarachnoid hemorrhage. In *Cerebral Arterial Spasm* (Wilkins RH, ed) Baltimore/London, Williams & Wilkins, pp 496-498
- Peerless SJ, Fox AJ, Komatsu K, Hunter IG (1982) Angiographic study of vasospasm following subarachnoid hemorrhage in monkeys. *Stroke* 13:473-479
- Peerless SJ, Kendall MJ (1975) Experimental cerebral vasospasm. In: *Proceedings of the Ninth Princeton Conference on Cerebral Vascular Disease* (Whisnant JP, Sandok BA, eds) New York, Grune & Stratton, pp 49-58
- Peterson EW, Choo SH, Lewis AJ, Lach B, Bormanis J (1980) Lysis of blood clot and experimental treatment of subarachnoid hemorrhage. In *Cerebral Arterial Spasm* (Wilkins RH, ed) Baltimore/London, Williams & Wilkins, pp 625-627
- Petruc KC, Weir BKA, Marriott MR, Overton TR (1973) Clinical grade, regional cerebral blood flow and angiographical spasm in the monkey after subarachnoid and subdural haemorrhage. *Stroke* 4:431-445
- Pickard JD, Boisvert PJ, Graham DI, Fitch W (1979) Late effects of subarachnoid haemorrhage on the response of the primate cerebral circulation to drug-induced changes in arterial blood pressure. *J Neurol Neurosurg Psychiatry* 42:899-903
- Pickard JD, Matheson M, Patterson J, Wyper D (1980) Prediction of late ischemic complications after cerebral aneurysm by the intraoperative measurement of cerebral blood flow. *J Neurosurg* 53:305-308

- Powers WJ, Grubb RL, Baker RP, Mintun MA, Raichle MR (1985) Regional cerebral blood flow and metabolism in reversible ischemia due to vasospasm. *J Neurosurg* 62:539-546
- Quintana L, Konda R, Ishibashi Y, Yoshimota T, Suzuki J (1982) The effect of prostacyclin on cerebral vasospasm. An experimental study. *Acta Neurochir* 62:187-193
- Raynor RB, McMurtry JG, Pool JL (1961) Cerebrovascular effects of topically applied serotonin in the cat. *Neurology (Minneapolis)* 11:190-195
- Recht LD, Nilaver G, Abrams GM, Haldar J, Zimmerman EA (1982) Oxytocin innervation of cerebral blood vessels: a possible role in local vasoregulation. *Neurology* 32 (2):A 110
- Ricardo JA, Koh ET (1978) Anatomical evidence of direct projections from the nucleus of the solitary tract to the hypothalamus, amygdala and other forebrain structures in the rat. *Brain Res.* 153:1-26
- Ritchie WL, Overton TR, Weir BKA (1980) Treatment of the ischemic complications after subarachnoid hemorrhage with specific reference to the use of hypertension and intravascular volume expansion. In *Cerebral Arterial Spasm* (Wilkins RH, ed) Baltimore/London, Williams & Wilkins, pp 673-679
- Robertson EG (1949) Cerebral lesions due to intracranial aneurysms. *Brain* 72:150-185
- Sahlin Ch, Brismar J, Delgado T, Owman Ch, Salford LG, Svendgaard NAa (1986) Cerebrovascular and metabolic changes during the delayed vasospasm following experimental subarachnoid hemorrhage in baboons, and treatment with a calcium antagonist. Submitted for publication.
- Saito I, Shigeno T, Aritake K, Tanishima T, Sano K (1979) Vasospasm assessed by angiography and computerized tomography. *J Neurosurg* 51:466-475
- Saito I, Ueda Y, Sano K (1977) Significance of vasospasm in the treatment of ruptured intracranial aneurysms. *J Neurosurg* 47:412-429
- Sakurada I, Kennedy C, Jehle J, Brown JD, Carbin GL, Sokoloff LC (1978) Measurement of local cerebral blood flow with iodo-¹⁴C antipyrine. *Am J Physiol* 234 (1):H59-H66
- Sano K, Saito I (1978) Timing and indication of surgery for ruptured intracranial aneurysms with regard to cerebral vasospasm. *Acta Neurochir* 41:49-60
- Sano K, Saito I (1980) Early operation and wash out of blood clots for prevention of cerebral vasospasm. In *Cerebral Arterial Spasm* (Wilkins RH, ed) Baltimore/London, Williams & Wilkins, pp 510-513
- Sarkar DK, Smith GC, Fink G (1981) Effect of manipulating central catecholamines on puberty and the surge of luteinizing hormone and gonadotropin releasing hormone induced by pregnant mare serum gonadotropin in female rats. *Brain Res* 213:335-349
- Sasaki T, Asano T, Sano K (1980) Cerebral vasospasm and free radical reactions. *Neurol Med Chir (Tokyo)* 20:145-153
- Sasaki T, Murota S, Wakai S, Asano T, Sano K (1981) Evaluation of prostaglandin biosynthetic activity in canine basilar artery following subarachnoid injection of blood. *J Neurosurg* 55:771-778
- Sasaki T, Wakai S, Asano T, Takakura K, Sano K (1982) Prevention of cerebral

- vasospasm after SAH with a thromboxane synthetase inhibitor, OKY-1581. *J Neurosurg* 57:74-82
- Savaki HE, Graham DI, Grome JJ, McCulloch J (1984) Functional consequences of unilateral lesion of the locus coeruleus: a quantitative ^{14}C 2-deoxyglucose investigation. *Brain Res* 292:239-249
- Sawchenko PE, Swanson LW (1981) Central noradrenergic pathways for the integration of hypothalamic neuroendocrine and autonomic responses. *Science* 214:685-687
- Schwartz WT (1978) 6-hydroxydopamine lesions of rat locus coeruleus alter brain glucose consumption, as measured by the 2-deoxy-D-(^{14}C) glucose tracer technique. *Neurosci Lett* 7:141-150
- Shiobara R, Kawase T, Toya S, Ebato K, Miyahara Y (1985) "Scavengery surgery" for subarachnoid haemorrhage (II) Continuous ventriculo-cisternal perfusion using artificial cerebrospinal fluid with urokinase. In *Timing of Aneurysm Surgery* (Auer LM, ed) Berlin/New York, deGruyter, pp 365-372
- Simeone FA, Peerless SJ (1975) Prolonged cerebral vasospasm without subarachnoid hemorrhage. In *Subarachnoid Hemorrhage and Cerebrovascular Spasm* (Smith RR, Robertson JT, eds) Springfield, Charles C Thomas, pp 206-225
- Simeone FA, Trepper PJ, Brown DJ (1972) Cerebral blood flow evaluation of prolonged experimental vasospasm. *J Neurosurg* 37:302-311
- Simeone FA, Vinall PE (1980) Effect of oxygen and glucose deprivation on vasoactivity and calcium movement in isolated middle cerebral arteries. In *Cerebral Arterial Spasm* (Wilkins RH, ed) Baltimore/London, Williams & Wilkins, pp 284-286
- Smith B (1963) Cerebral pathology in subarachnoid haemorrhage. *J Neurol Neurosurg Psychiatry* 26:535-539
- Smith GC, Courtney PG, Wreford NGM, Walker MMcd (1982) Further studies on the effects of intravenously administered 6-hydroxydopamine on the median eminence of the rat. *Brain Res* 234:101-110
- Sokoloff L, Reivich M, Kennedy C, Des Rosiers MH, Patlak CS, Pattigrew KD, Sakurada O, Shinohara M (1977) The ^{14}C deoxyglucose method for the measurement of local cerebral glucose utilization: theory, procedure and normal values in the conscious and anesthetized albino rat. *J Neurochem* 28:897-917
- Sundt TM Jr (1980) The role of isoproterenol in the treatment of vasospasm. In *Cerebral Arterial Spasm* (Wilkins RH, ed) Baltimore/ London, Williams & Wilkins, pp 572-574
- Sundt TM Jr, Kobayashi S, Fobe NC (1982) Results and complications of surgical management of 809 intracranial aneurysms in 722 patients. *J Neurosurg* 56:753-765
- Sundt TM Jr, Onofrio BM, Merideth J (1973) Treatment of cerebral vasospasm from subarachnoid hemorrhage with isoproterenol and lidocaine hydrochloride. *J Neurosurg* 38:557-560
- Suwanwela C, Suwanwela N (1972) Intracranial arterial narrowing and spasm in acute head injury. *J Neurosurg* 36:314-323
- Suzuki J, Yoshimoto T (1979) The effect of mannitol in prolongation of permissible occlusion time of cerebral artery — clinical data of aneurysm surgery. *Neurosurg Rev* 1:13-19

- Svendgaard NAa, Brismar J, Delgado TJ, Diemer NH (1985) The effect on the development of cerebral vasospasm in the rat of lesioning of the peripheral and central catecholamine systems. *Neurol Res* 7:30-34
- Svendgaard NAa, Brismar J, Delgado T, Egund N, Owman Ch, Rodacki MA, Sahlin Ch, Salford LG (1983) Late cerebral arterial spasm: the cerebrovascular response to hypercapnia, induced hypertension and the effect of nimodipine on blood flow autoregulation in experimental subarachnoid hemorrhage in primates. *Gen Pharmac* 14:167-172
- Svendgaard NAa, Edvinsson L, Olin T, Owman Ch, Sahlin Ch (1977) On the pathophysiology of cerebral vasospasm: transmitter changes in perivascular sympathetic nerves, and increased pial artery sensitivity to norepinephrine and serotonin. In *Neurogenic Control of Brain Circulation. Proceedings of International Symposium Wenner-Gren* (Owman Ch, Edvinsson L, eds) Oxford/New York, Pergamon Pres, pp 143-152
- Tanabe Y, Sakata K, Yamada H, Ito T, Takada MC (1978) Cerebral vasospasm and ultrastructural changes in cerebral arterial wall. *J Neurosurg* 49:229-238
- Tanaka K, Gotoh F, Muramatsu F, Fukuuchi Y, Okayasu H, Suzuki N, Kobari M (1982) Effect of nimodipine, a calcium antagonist, on cerebral vasospasm after subarachnoid hemorrhage in cats. *Drug Res* 32:1529-1534
- Tani E, Maeda Y, Fukumori T, Nakano M, Kochi N, Morimura T, Yokota M, Matsumoto T (1984) Effect of selective inhibitor of thromboxane A_2 synthetase on cerebral vasospasm after early surgery. *J Neurosurg* 61:24-29
- Tani E, Yamagata S, Ito Y (1978) Intercellular granules and vesicles in prolonged cerebral vasospasm. *J Neurosurg* 48:179-189
- Toda N, Shimizu K, Ohta T (1980) Mechanism of cerebral arterial contraction induced by blood constituents. *J Neurosurg* 53:312-322
- Tranzer JP, Thoenen HC (1967) Ultramorphologische Veränderungen der Sympathischen Nervenendigungen der Katze nach Vorbehandlung mit 5- und 6-Hydroxydopamine. *Naunyn Schmiedebergs Arch Pharmac Exp Path* 257:343-344
- Trump BF, Berezesky IK, Laiho KU, Osornio AR, Mergner WJ, Smith MW (1980) The role of calcium in cell injury. A review. In *Scanning Electron Microscopy II* (O'Hare AMF, ed) Chicago, SEM Inc, pp 437-462
- Ungerstedt U (1971) Effect of 6-hydroxydopamine on the monoamine-containing neurons in the CNS. Histochemical studies on the effect of intracerebral and intraventricular injections of 6-hydroxydopamine on monoamine neurons in the rat brain. In *6-Hydroxydopamine and Catecholamine Neurons* (Malmfors T, Thoenen H, eds) Amsterdam, North Holland, pp 101-127
- Varsos VG, Liszczak TM, Han DH, Kistler JP, Vielma J, Black PMCL, Heros RC, Zervas NT (1983) Delayed cerebral vasospasm is not reversible by aminophylline, nifedipine, or papaverine in a "two-hemorrhage" canine model. *J Neurosurg* 58:11-17
- Voldby B, Enevoldsen EM, Jensen FT (1985a) Regional CBF, intraventricular pressure, and cerebral metabolism in patients with ruptured intracranial aneurysms. *J Neurosurg* 62:48-58
- Voldby B, Enevoldsen EM, Jensen FT (1985b) Cerebrovascular reactivity in patients with ruptured intracranial aneurysms. *J Neurosurg* 62:59-67
- Voldby B, Petersen OF, Buhl M, Jakobsen P, Østergaard R (1984) Reversal of

- cerebral arterial spasm by intrathecal administration of a calcium antagonist (nimodipine). *Acta Neurochir* 70:243-254
- Weir B, Grace M, Hansen J, Rothberg C (1978) Time course of vasospasm in man. *J Neurosurg* 48:173-178
- Weir B, Rothberg C, Grace M, Davis F (1975) Relative prognostic significance of vasospasm following subarachnoid hemorrhage. *Can J Neurol Sci* 2:109-114
- Wellum GR, Zervas NT, Irvine TW (1980) Effect of oxyhemoglobin on the contractility of in vitro arterial smooth muscle. In *Cerebral Arterial Spasm* (Wilkins RH, ed) Baltimore/London, Williams & Wilkins, pp 173-180
- Westerberg V, Lewis J, Corcoran ME (1984) Depletion of noradrenaline fails to affect kindled seizures. *Exp Neurol* 84:237-240
- White RP, Chapleau CE, Dugdale M, Robertson JT (1980) Cerebral arterial contractions induced by human and bovine thrombin. *Stroke* 11:363-368
- White RP, Hagen AA, Morgan H, Dawson WN, Robertson JT (1975) Experimental study on the genesis of cerebral vasospasm. *Stroke* 6:52-57
- White RP, Robertson JT (1983) Comparison of piroxicam, meclofenamate, ibuprofen, aspirin, and prostacyclin efficacy in a chronic model of cerebral vasospasm. *Neurosurgery* 12:40-46
- White RP, Robertson JT (1985) Role of plasmin, thrombin, and antithrombin III as etiological factors in delayed cerebral vasospasm. *Neurosurgery* 16:27-35
- Wilkins RH (1975) Intracranial vascular spasm in head injuries. In *Handbook of Clinical Neurology*, Vol 23, *Injuries of the Brain and Skull, Part I* (Vinken PJ, Bruyn GW, Braakman R, eds) Amsterdam, North Holland, pp. 163-197
- Wilkins RH (1980) Attempted prevention or treatment of intracranial arterial spasm: a survey. *Neurosurgery* 6: 198-210
- Wilkins RH, Odom GL (1970) Intracranial arterial spasm associated with craniocerebral trauma. *J Neurosurg* 32:626-633
- Wood JH, Simeone FA, Kron RE, Litt M (1982) Rheological aspects of experimental hypervolemic hemodilution with low molecular weight dextran: relationship of cortical blood flow, cardiac output, and intracranial pressure to fresh blood viscosity and plasma volume. *Neurosurgery* 11:739-753
- Zervas NT, Candia M, Candia G, Kido D, Pessin MS, Rosoff CB, Bacon V (1979) Reduced incidence of cerebral ischemia following rupture of intracranial aneurysms. *Surg Neurol* 11:339-344
- Zervas NT, Hori H, Rosoff CB (1974) Experimental inhibition of serotonin by antibiotic: Prevention of cerebral vasospasm. *J Neurosurg* 41:59-62
- Zervas NT, Kuwayama A, Rosoff CB, Salzman EW (1973) Cerebral arterial spasm: Modification by inhibition of platelet function. *Arch Neurol* 28:400-404

Subarachnoid haemorrhage in the rat: angiography and fluorescence microscopy of the major cerebral arteries

Tia Juana Delgado, M.D., Jan Brismar*, M.D., Niels Aage Svendgaard, M.D.

*Neurosurgical Research Department, *Department of Neuroradiology, University Hospital, Lund, Sweden*

SUMMARY

A subarachnoid haemorrhage (SAH) in the rat was produced by the injection of blood via a previously implanted catheter connected to the cisterna magna. Repeated angiographical examinations of the vertebro-basilar arteries revealed a biphasic vasospasm with a maximal acute spasm at ten minutes and a maximal late spasm at two days after cisternal blood injection. Fluorescence microscopical examination of the major cerebral arteries at day two after the SAH revealed a reduction in the fluorescence intensity and in the number of histochemically visible sympathetic nerve terminals.

INTRODUCTION

Cerebral vasospasm is an angiographically demonstrable, variable arterial constriction that can be clinically asymptomatic or give rise to increasing neurological deficits or death. Vasospasm frequently occurs following a subarachnoid haemorrhage (SAH) secondary to rupture of an intracranial aneurysm. The mechanism underlying the development of spasm is not known.

Research has focused on the discovery of a spasmogenic factor. Monoamines^{1, 2, 3, 4, 5, 6} and prostaglandins^{7, 8, 9} have been proposed as spasm inducing factors. Breakdown products of erythrocytes^{10, 11, 12} and free radicals^{13, 14} have also been suggested. Correspondingly, monoamine antagonists or inhibitors have been used to prevent or treat vasospasm^{15, 16, 17, 18, 19}. The effect of thromboxane synthetase inhibitors and prostacyclin on spasm has also been investigated^{20, 21}. However, there is still no definitive treatment for spasm and the underlying mechanism remains unknown.

In our opinion, there is a need for a simple and inexpensive SAH model giving a reproducible vasospasm in the investigation of the mechanism underlying the spasm syndrome. Therefore, we have developed a SAH model in the rat. This communication presents the angiographical data, and the results of the fluorescence microscopical examinations of the major cerebral arteries following a SAH.

MATERIAL AND METHODS

Eighty-seven Sprague-Dawley rats weighing between 300 and 425 g were used.

ANIMAL PREPARATIONS

Stage I. The animals were anaesthetized with chloral hydrate (250 mg/kg i.p.). An opaque x-ray catheter was inserted into the cisterna magna for subsequent injection of blood. The proximal part of the catheter was blunted and attached to the atlanto-occipital membrane with a purse string suture. The distal tip of the catheter was sealed and sutured subcutaneously to an external skull muscle.

Stage II. Three to seven days after the implantation of the cisternal catheter, the animals were prepared for angiography. The anaesthesia was initiated with 4% halothane. The trachea was exposed and a tube (premature Infant feeding tube size 8—CR Bard International Ltd., England) was inserted orally and observed entering the trachea. Respiration was controlled in a semiopen circuit using a servoventilator (manufactured at the University Hospital, Lund, Sweden). Anaesthesia was maintained with 70% nitrous-oxide and 30% oxygen.

Catheters were inserted into a femoral artery and vein for continuous blood pressure monitoring and for infusion of drugs, respectively. During the surgical procedure, 0.75% halothane was added to the gas mixture. Heparin (Vitrum, 75 IU/kg) was given intravenously.

The angiography catheter was inserted into the axillary artery bilaterally. By elevating the pectoral muscle, three branches of the brachial-axillary artery were identified. The two proximal branches (deep brachial artery, circumflex-subscapular trunk) were coagulated to reduce the distribution area of the contrast. The distal branch (superficial radial artery) was left intact. The catheter was inserted distal to this branch. The catheter was made of radiopaque polyethylene (Surgimed A/S, Denmark) and the dimensions were OD/ID=0.9/0.5 mm. The tip of the catheter was tapered to an outer diameter of approximately 0.4 mm. The catheter tip was placed about two mm distal to the origin of the vertebral artery. After the surgical procedure, the halothane was switched off and the animal was placed in a prone position on a plexiglass plate. Suxamethonium chloride (Celocurin, Vitrum; 3mg/kg) was given and half an hour was allowed to pass before the angiography. Blood samples were drawn periodically for measurement of pH, paO_2 and paCO_2 .

Following the control angiography, 0.07 or 0.3 ml homologous blood was injected into the cisterna magna via the subcutaneous

catheter over about five or 30 seconds, respectively. Before the blood injection, cerebrospinal fluid (about 0.03 ml) was gently aspirated. During the injection of blood, the head was lowered by tilting the animal 20°. A transitory increase in blood pressure of five to ten mmHg lasting about ten seconds occurred after injection of 0.07 ml blood. Similarly, injection of 0.3 ml blood caused a transitory increase in blood pressure of 20-30 mmHg lasting about 60 seconds. There was also a decrease in pulse rate in the two groups. Angiography was done at predetermined time points following the cisternal injection (see experimental groups). After the angiography, the catheters were removed and the axillary arteries were ligated distal to the superficial radial artery. The animals were extubated when they were fully awake.

Stage III. One to seven days later, the animals were again anaesthetized and intubated as described earlier. Catheters were reinserted in the axillary and femoral vessels. The angiography was repeated. In addition to the control angiography and one or two examinations during a 90 minute period after the blood injection, most of the animals were reexamined once between day one and day seven after the SAH. A minor group of animals was examined twice between day one and day seven. After the final examination, the animals were sacrificed.

ANGIOGRAPHY

An x-ray tube (Opti-100/12/15 HSG, Elema-Siemens, Sweden) with a 0.2×0.2 mm focus spot was used; exposure data were 80 mAs, 60 kV and 0.16 sec. The focus-objective distance was 43 cm, and the focus-film distance was 123 cm, giving a linear magnification of 2.86. Angiograms were made on mammographic film (Kodak NMB Film).

For angiography, metrizamide (Amipaque®, Nyegaard and Co., Oslo, Norway), in a concentration of 370 mg I/ml, was simultaneously manually injected into both axillary catheters over approximately three seconds. The total amount of contrast medium injected was 0.7 ml; 0.4 ml on the right and 0.3 ml on the left side. Before injection of contrast, one film was exposed for subtraction. Amipaque was chosen because of its low toxicity; it is nonionic with an osmolality of about one-third that of conventional media, and has less vasodila-

tory effect than other media ²². All the angiographies were performed by the same investigator.

MEASUREMENT OF ARTERIAL DIAMETRE

Measurements of the vertebral and basilar arterial diametres before and after subarachnoid blood injection were made using a technique similar to that described by Gabrielsen and Greitz²³. Using a magnifying glass, the contour of the vessels was enhanced with a sharp soft pencil. The diametre of the vessel was then measured with a precision calibrated measuring lens of fixed magnification and lens film distance. A vascular index representing the mean value of the diametres at four preselected points within the vertebro-basilar system was calculated in an animal both before and after the SAH (Fig 1). The degree of vasoconstriction was determined from the ratio of the two indices. The measurements were made in random order without previous knowledge of whether or not the animals had been injected with blood.

FLUORESCENCE MICROSCOPY

Under barbiturate anaesthesia (Brietal, Lilly; 40mg/kg i.p.) vascular perfusion was done with isotonic cold saline. Following brain removal, the major cerebral arteries were extirpated and briefly rinsed in cold saline. Whole-mount preparations of vertebral, basilar, internal carotid and middle cerebral arteries were dried *in vacuo* in a dessicator over phosphorous pentoxide for at least two hours. They were exposed to formaldehyde gas of optimal humidity at 80°C for one hour, based on the method described by Falck et al.²⁴. (for details of the present technique, see Björklund et al., 1972²⁵). The preparations were mounted in liquid paraffin and examined in a Zeiss fluorescence microscope equipped with BG 12 (Schott) as mercury lamp filter and Zeiss 47 + 50 as secondary filters.

EXPERIMENTAL GROUPS

In four animals, blood mixed with cardiac green was injected into the cisternal catheter to determine the distribution of blood



Fig 1. Bilateral vertebral angiography in the rat showing the points (small arrows) at which vertebral and basilar artery diameters were measured: 0.5 cm proximal to the vertebro-basilar junction on the vertebral arteries; on the basilar artery 0.5 cm above the junction of the vertebral arteries and 0.5 cm below the origin of the posterior cerebral arteries. An index was calculated as the mean value of the measured diameters. Axillary catheters are indicated by large arrows. Axial view, magnification 2.86.

within the subarachnoid space. The animals were sacrificed right after the injection.

In five normal animals, repeated angiography was done to investigate the spontaneous intra-individual variations in vessel diameter. The animals had angiography twice on day zero. They were reexamined once during the following week.

Fifty-two animals were used to delineate the degree and duration of vasoconstriction following the injection of 0.3 ml blood intracisternally. A control vertebro-basilar angiography was done in all animals before blood injection. Five to ten animals were reexamined at each of the following time points: 5, 10, 15, 30, 60 and 90 minutes and 1, 2, 3, 5, and 7 days following the blood injection.

Six animals had a control angiography followed by intracisternal injection of 0.07 ml of blood. Repeat angiography was performed at ten minutes and two days post SAH.

Six animals had a control angiography followed by intracisternal injection of 0.3 ml of saline. Repeat angiography was performed at ten minutes and/or two days after the injection.

Eight animals had intracisternal injection of 0.3 ml blood and no angiography. The vertebral, basilar, internal carotid and the middle cerebral arteries were dissected out two days later and processed for fluorescence microscopy. Three animals had intracisternal injection of 0.3 ml saline. Two days later, the cerebral arteries were processed for fluorescence microscopy.

RESULTS

The distribution in the subarachnoid space of the blood-cardiac green mixture showed a consistent pattern. There was a marked green staining of the entire basal surface of the brain. There were also several patchy areas of staining over the hemispheres.

The physiological parameters before and after cisternal injection are given in Tables I and II. The values for 30, 60 and 90 minutes and five days after a cisternal blood injection are not shown in Table I. The values at these time points did not differ from values obtained at the other time points. The temperatures of the animals were kept close to 37°. The paCO_2 values were around 37 mm Hg, the paO_2 values were about 150 mm Hg, and the pH was close to 7.4.

There was a good and reproducible filling of the vertebro-

TABLE I

Physiological parameters in animals with a cisternal injection of 0.3 ml blood

TIME	MABP (mmHg)	PULSE	pH	$P_{a}O_2$ (mmHg)	$P_{a}CO_2$ (mmHg)	n
control	121 $\pm$ 6	324 $\pm$ 15	7.43 $\pm$ 0.01	153 $\pm$ 7	38.2 $\pm$ 1.3	7
5 minutes	134 $\pm$ 6	289 $\pm$ 13	7.43 $\pm$ 0.01	153 $\pm$ 7	38.2 $\pm$ 1.3	7
control	125 $\pm$ 4	315 $\pm$ 20	7.37 $\pm$ 0.03	151 $\pm$ 8	37.3 $\pm$ 1.1	10
10 minutes	128 $\pm$ 7	289 $\pm$ 23	7.37 $\pm$ 0.03	151 $\pm$ 8	37.3 $\pm$ 1.1	10
control	127 $\pm$ 6	324 $\pm$ 25	7.42 $\pm$ 0.03	146 $\pm$ 19	37.4 $\pm$ 1.0	8
15 minutes	126 $\pm$ 3	323 $\pm$ 28	7.38 $\pm$ 0.03	149 $\pm$ 16	38.3 $\pm$ 1.3	8
control	124 $\pm$ 8	309 $\pm$ 13	7.42 $\pm$ 0.03	170 $\pm$ 11	37.0 $\pm$ 2.0	5
day 1	106 $\pm$ 9	314 $\pm$ 27	7.37 $\pm$ 0.01	172 $\pm$ 7	37.3 $\pm$ 1.6	5
control	123 $\pm$ 4	300 $\pm$ 8	7.41 $\pm$ 0.02	166 $\pm$ 4	36.5 $\pm$ 1.3	6
day 2	113 $\pm$ 7	290 $\pm$ 20	7.45 $\pm$ 0.02	167 $\pm$ 18	34.9 $\pm$ 0.7	6
control	117 $\pm$ 10	340 $\pm$ 18	7.41 $\pm$ 0.02	168 $\pm$ 10	37.4 $\pm$ 1.2	5
day 3	112 $\pm$ 4	293 $\pm$ 23	7.42 $\pm$ 0.03	156 $\pm$ 11	39.7 $\pm$ 3.0	5
control	130 $\pm$ 5	300 $\pm$ 23	7.42 $\pm$ 0.01	161 $\pm$ 10	39.0 $\pm$ 0.9	7
day 7	121 $\pm$ 5	300 $\pm$ 16	7.40 $\pm$ 0.05	157 $\pm$ 18	38.2 $\pm$ 1.2	7

MABP = Mean Arterial Blood Pressure

The values are means $\pm$ S.E.M.

TABLE II

Physiological parameters in animals with a cisternal injection of 0.3 ml saline and 0.07 ml blood

TIME	MABP (mmHg)	PULSE	pH	$P_{a}O_2$ (mmHg)	$P_{a}CO_2$ (mmHg)	N
control	116 $\pm$ 8	295 $\pm$ 13	7.40 $\pm$ 0.03	165 $\pm$ 3	36.6 $\pm$ 2.1	3
10 minutes after saline injection	131 $\pm$ 5	305 $\pm$ 13	7.40 $\pm$ 0.03	165 $\pm$ 3	36.6 $\pm$ 2.1	3
control	119 $\pm$ 7	285 $\pm$ 15	7.42 $\pm$ 0.02	150 $\pm$ 11	36.3 $\pm$ 1.5	5
day 2 after saline injection	112 $\pm$ 5	319 $\pm$ 17	7.42 $\pm$ 0.02	148 $\pm$ 9	38.0 $\pm$ 1.8	5
control	125 $\pm$ 5	336 $\pm$ 26	7.44 $\pm$ 0.03	156 $\pm$ 13	35.4 $\pm$ 1.6	6
10 minutes after blood injection	127 $\pm$ 7	285 $\pm$ 15	7.44 $\pm$ 0.03	156 $\pm$ 13	35.4 $\pm$ 1.6	6
control	125 $\pm$ 5	336 $\pm$ 26	7.44 $\pm$ 0.03	156 $\pm$ 13	35.4 $\pm$ 1.6	6
day 2 after blood injection	110 $\pm$ 9	282 $\pm$ 32	7.44 $\pm$ 0.04	150 $\pm$ 17	35.1 $\pm$ 1.0	6

MABP = Mean Arterial Blood Pressure

The values are means $\pm$ S.E.M.



Fig 2. Vertebro-basilar angiography in the rat. A, control, B and C, ten minutes and five days after cisternal blood injection, respectively. There is spasm in the vertebro-basilar system at ten minutes. Spasm is also seen in the internal carotid system. Arrows indicate basilar artery.

basilar system with the axillary angiography. In a number of animals, there was also good filling of the right internal carotid artery (Fig. 1). In the five animals used to investigate intra-individual variations, the standard deviation of the mean vessel diameter of the vertebro-basilar system was 1.5%; the maximal difference was 4%.



Fig 3. Vertebro-basilar angiography in the rat. A, control, B and C, two and seven days, respectively, after blood injection. The spasm seen at day two is not seen at day seven. Arrows indicate basilar artery.

Cisternal injection of blood produced a constriction of the vertebro-basilar arteries in all the animals receiving 0.3 ml blood. Fig. 2 shows an angiography with spasm of the vertebro-basilar system ten minutes after cisternal blood injection. The spasm had disappeared by day five. Fig. 3 shows spasm at two days that had disappeared by day seven. There was a biphasic pattern of vasospasm (Fig.4). During the acute phase, there was a maximal mean degree of spasm of 40% occurring at ten minutes after blood injection. During the late

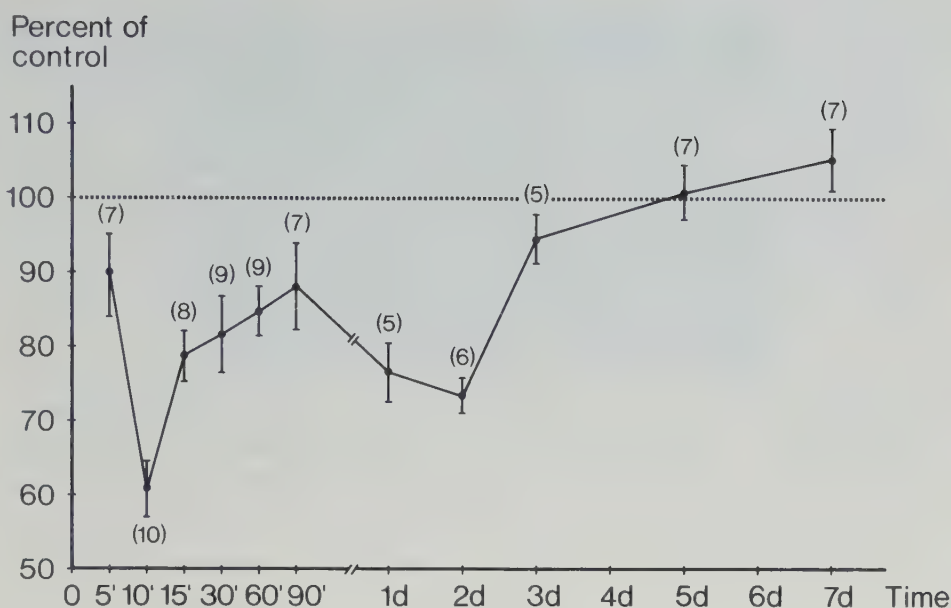


Fig 4. Changes in mean vertebro-basilar diameter (per cent of control $\pm$ SEM) at various time periods after intracisternal blood injection in the rat ; number of animals studied within brackets.

phase, there was a maximal mean degree of spasm of 27 % occurring at day two. A slight dilatation of the vessels was noted at day five and day seven. The spasm was diffuse in most of the animals. In a few animals, bead like irregularities on the narrowed vessels were seen during the late phase. The degree of vasoconstriction in the animals with cisternal injection of 0.07 ml blood was slightly less than that seen in the animals receiving 0.3 ml blood (mean vessel diameter in percent of control $\pm$ SEM: at ten minutes 66.4 ± 4.8 for animals receiving 0.07 ml blood versus 60.7 ± 3.8 for animals receiving 0.3 ml blood; at two days 77.5 ± 6.0 versus 73.1 ± 2.4). The six animals injected with saline did not develop vasoconstriction. (Mean vessel diameter in percent of control $\pm$ SEM, ten minutes: 103.7 ± 2.9 ; two days: 102 ± 2.8).

The blood injected animals were noticeably drowsy the first two-three days after the SAH. No paralysis was noted in any of the animals. There was a weight loss of about 10% during the first week after the SAH. The behavior of the saline injected animals was normal.

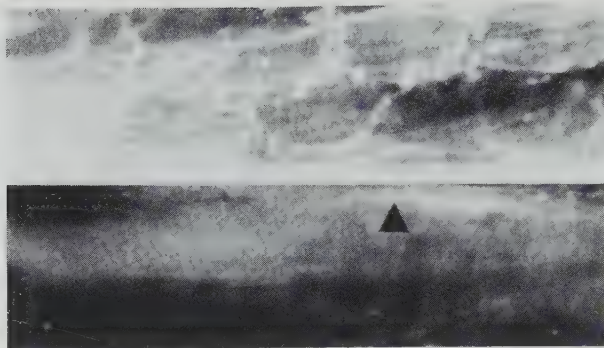


Fig 5. Whole-mount preparations from basilar arteries of the rat after formaldehyde treatment (80°C, 1 hour) for demonstration of noradrenergic fibers. Normal basilar artery from an animal with a typical sympathetic plexus (upper). Basilar artery from an animal two days after an intracisternal injection of blood (lower). The fluorescence intensity is markedly reduced in the very few remaining fibers (arrows). Magnification: 300X.

Six of the 52 animals undergoing cisternal blood injection and angiography died, i.e. a mortality of 11.5%. The main cause of death was respiratory failure secondary to obstruction of the tracheal tube. There was no mortality in the six animals receiving saline or in the animals receiving 0.07 ml blood.

Three animals developed an infection following the implantation of the cisternal catheter. They were not included in the experimental groups.

Gross pathological examination of the brains revealed a complete absence of cisternal blood by day three after the SAH. There was a xanthochromic discoloration of the basal surface of the brain that was most pronounced at day two and gradually disappeared over the following five days. There were no signs of brain stem damage secondary to the intracisternal catheter.

In the fluorescence microscopical examination of the cerebral arteries from the animals receiving intracisternal saline, the adrenergic transmitter in the sympathetic nerves was displayed as a bright yellow-green fluorescent network of beaded fibres (Fig. 5 upper). In the animals receiving intracisternal blood, there was a marked reduction in the fluorescence intensity and a substantial reduction in the number of histochemically visible nerve terminals (Fig. 5 lower).

DISCUSSION

The present study demonstrates that a SAH can be produced in the rat by injection of blood via a previously implanted catheter. The use of a catheter connected to the cisterna magna for blood injection abolishes the need for opening the skull that has been a common disadvantage in small animal SAH models^{26,27}. An open skull eliminates the effect that changes in intracranial pressure might have on spasm. There was no mortality associated with the implantation of the catheter, and the frequency of infections was negligible.

Angiography via bilateral axillary catheters gives a good visualization of the posterior cerebral circulation, and allows measurements of the vessel diameter at several preselected points within the vertebro-basilar system. However, the present angiographical technique does not give a consistent filling of the carotid system. The amount of contrast needed to obtain filling of both the carotid and the vertebral arteries would cause an undesirable rise in blood pressure and also a marked increase in mortality. Angiographical examination instead of direct visualization allows repeated examinations over a long time period, and a larger vascular area can be evaluated. It also circumvents the need for a craniotomy.

In the literature, there are different criteria for determining what degree of vasoconstriction in an experimental SAH model could be designated as vasospasm. Espinosa et al. considered a constriction of 10% as spasm²⁸. Peerless et al. used the term spasm for a vessel diameter reduction of more than 20% compared with the baseline angiogram²⁹. We describe a constriction surpassing 8% as vasospasm i.e. twice the maximal difference in the observed variation in mean vessel diameter.

The investigation has shown that there is a biphasic pattern of vasospasm in the rat. The duration of spasm was three days. It is noteworthy that the occurrence of spasm was limited to the period when blood was found in the subarachnoid space. A biphasic time course has been previously described in dogs^{30,31,32}, and in primates^{29,33,34}, but not in the rat. The late phase of spasm occurs earlier (on day two) in the rat as compared to other species. In the rabbit, the late spasm reaches a maximum three to five days after cisternal blood injection⁵. In the dog³⁰ and in the monkey^{28,35} a late spasm phase has been demonstrated with a maximum between the fifth and the seventh day after the SAH.

Clinically, the existence of an acute spasm phase has not definitely been proven. In patients, the late spasm appears angiographically two to four days after the haemorrhage and reaches a maximal around day seven. It is during the late phase that the ischemia and the neurological deficits develop.

The animals were apathetic for two to three days after the cisternal blood injection. No focal neurological deficits were noted in any of the animals. However, a neurological evaluation is difficult in small animals.

The animals receiving 0.07 ml blood intracisternally had less spasm than the group receiving 0.3 ml. Also, the reproducibility of the late spasm was somewhat less in the group receiving 0.07 ml; one of the animals in this group did not have spasm at two days. Although a greater difference in the degree of spasm could have been expected, the data are in keeping with findings from both clinical and experimental investigations that a larger SAH increases the degree and the probability of developing vasospasm^{36,28}.

The degree and duration of increase in blood pressure following intracisternal injection of 0.07 ml or 0.3 ml blood, indicate that there is a substantial difference in the intracranial pressure between the two groups. The minor difference in spasm in the two groups therefore suggests that it is the blood per se and not the combination of blood and a marked increase in intracranial pressure that induces the spasm.

The fluorescence microscopical examination showed changes in the perivascular sympathetic nerves similar to the changes noted in vessels from monkeys³⁷, rabbits⁵ and cats³⁸ after a SAH.

Barry et al.²⁶ induced vasospasm in Sprague-Dawley rats by puncturing the basilar artery via a transcervical and transclival approach. The basilar artery diameter was measured photographically. The animals were examined on two separate occasions over a seven day period. Similar to our findings, the spasm peak was seen on day two. The acute phase was not documented in their series. Ohta et al.²⁷ using a similar approach and photographic technique as that of Barry et al.²⁶, induced vasospasm in Wistar rats by irrigating the basilar artery with barium chloride. In a few animals, whole blood or blood components were used to induce spasm. The observation time was six hours. Whole blood produced a severe vasospasm that disappeared spontaneously within 30 minutes. In a comparative

study between Wistar and Sprague-Dawley rats, we have found that vasospasm is less pronounced and of shorter duration in Wistar rats (unpublished observations).

Fein³⁹ induced a SAH in Sprague-Dawley rats via the injection of whole blood into the cisterna magna. However, the objective of his study was to investigate the acute metabolic changes after a SAH.

Bouillin et al⁴⁰ presented an angiographical model in the rat for studying the aetiology of cerebral arterial constriction. The angiography was performed via catheterization and ligation of one common carotid artery.

In conclusion: The present investigation has demonstrated that a reproducible biphasic vasospasm can be induced in the rat and evaluated with repeated angiographical examinations. The model can be used to investigate spasm induced ischemia with cerebral blood flow and metabolic studies. Moreover, the model is suitable in the study of the basic mechanism of vasospasm and in therapeutic trials.

ACKNOWLEDGEMENT

This study was supported by grants from the Swedish Medical Research Council (No. B88-04X-05300-03), Einar Björkelund Foundation, Anna Lisa and Sven-Eric Lundgren Foundation and Thorsten and Elsa Segerfalk Foundation.

REFERENCES

1. Alksne JF, Greenhoot JH: Experimental catecholamine-induced chronic cerebral vasospasm. Myonecrosis in vessel wall. *J Neurosurg* 41:440-445, 1974
2. Chow RWB, Newton TH, Smith MC, Adams JE: Cerebral vasospasm induced by subarachnoid blood and serotonin: an angiographical study. *Invest Radiol* 3:402-407, 1968
3. Peerless SJ, Kendall MJ: Experimental cerebral vasospasm. In: *Proceedings of the Ninth Princeton Conference on Cerebral Vascular Disease*. Whistnant JP & Sandok BA (eds), New York, Grune and Stratton, pp. 49-58, 1974
4. Raynor RB, McMurtry JG, Pool JL: Cerebrovascular effects of topically applied serotonin in the cat. *Neurology (Minneapolis)* 11:190-195, 1961
5. Svendgaard NA, Edvinsson L, Olin T, Owman Ch, Sahlin Ch: On the pathophysiology of cerebral vasospasm: transmitter changes in perivascular sympathetic nerves, and increased pial artery sensitivity to norepinephrine and serotonin. In: *Neurogenic Control of Brain Circulation. Proceedings of International Symposium Wenner-Gren, Owman CH, Edvinsson L (eds), Oxford, New York, Pergamon Press, 1977, pp. 143-152*

6. White RP, Hagen A, Robertson JT: Experimental evaluation of the spasmogenicity of dopamine on the basilar artery. *J Neurosurg* 44:45-49, 1976
7. Boullin DJ: Cerebral vasospasm. Chichester, New York, John Wiley and Sons, 1980
8. Morgan H, White RP, Pennink M, Robertson JT: Prostaglandins and experimental cerebral vasospasm. *Surg Forum* 23:447-448, 1972
9. Pennink M, White RP, Crockarell JR, Robertson JT: Role of prostaglandin F_{2a} in the genesis of experimental cerebral vasospasm. Angiographic study in dogs. *J Neurosurg* 37:398-406, 1972
10. Ohta T, Kajikawa H, Yoshikawa J, et al: Cerebral vasospasm and hemoglobins: Clinical and experimental studies. In: *Cerebral Arterial Spasm. Proceedings of the 2nd International Workshop*, Amsterdam, Wilkins RH (ed), Baltimore, Williams and Wilkins, 1980, pp. 166-172
11. Osaka K: Prolonged vasospasm produced by the breakdown products of erythrocytes. *J Neurosurg* 47:403-411, 1977
12. Wellum GR, Zervas NT, Irvine TW: Effect of oxyhemoglobin on the contractility of in vitro arterial smooth muscle. In: *Cerebral Arterial Spasm. Proceedings of the 2nd International Workshop*, Amsterdam, Wilkins RH (ed), Baltimore, Williams and Wilkins, 1980, pp. 173-180
13. Asano T, Tanishima T, Sasaki T, Sano K: Possible participation of free radical reactions initiated by clot lysis in the pathogenesis of vasospasm after subarachnoid haemorrhage. In: *Cerebral Arterial Spasm. Proceedings of the 2nd International Workshop*, Amsterdam, Wilkins RH (ed) Baltimore, Williams and Wilkins, 1980, pp. 190-201
14. Sasaki T, Asano T, Sano K: Cerebral vasospasm and free radical reactions. *Neurol Med Chir*:145-153, 1980
15. Allen GS, Gold HHA, Chow SN, French LA: Cerebral arterial spasm. III. Intracisternal production of spasm by serotonin and blood and its reversal by phenoxybenzamine. *J Neurosurg* 40: 451-458, 1974
16. Cummins BH, Griffith HB: Intracarotid phenoxybenzamine for cerebral arterial spasm. *Brit Med J*, 1:382-383, 1971
17. Sundt TM Jr, Onofrio BM, Meredith J: Treatment of cerebral vasospasm from SAH with isoproterenol and lidocaine hydrochloride. *J Neurosurg* 38:557-560, 1973
18. Zervas NT, Hori H, Rosoff CB: Inhibition of serotonin by antibiotic and prevention of cerebral vasospasm. *J Neurosurg* 41:59-62, 1974
19. Zervas NT, Kuwayama A, Rosoff CB, Salzman EW: Cerebral arterial spasm. Modification in inhibition of platelet function. *Arch Neurol* 28:400-404, 1973
20. Quintana L, Konda R, Ishibashi Y, Yoshimota T, Suzuki J: The effect of prostacyclin on cerebral vasospasm. An experimental study. *Acta Neurochir* 62:187-193, 1982
21. Sasaki T, Wakai S, Asano T, Takakura K, Sano K: Prevention of cerebral vasospasm after SAH with a thromboxane synthetase inhibitor. OKY-1581. *J Neurosurg* 57: 74-82, 1982
22. Almén T, Boijesen E, Lindell S: Metrizamide in angiography. *Acta Radiol (Diagn.)* 18:33-38, 1977

23. Gabrielsen TO, Greitz T: Normal size of the internal carotid, middle cerebral and anterior cerebral arteries. *Acta Radiol (Diagn)* 10:1-10, 1970
24. Falck B, Hillarp NA, Thieme G, Torp A (1962): Fluorescence of catecholamines and related compounds condensed with formaldehyde. *J Histochem Cytochem* 10:348-354, 1962
25. Björklund A, Falck B, Owman Ch: Fluorescence microscopic and microspectrofluorometric techniques for the cellular localization and characterization of biogenic amines. In: *The Thyroid and Biogenic Amines, Methods of Investigative and Diagnostic Endocrinology*, Vol. 1, Rall JE, Kopin IJ (eds), Amsterdam, North Holland Publishing Co, 1972 pp. 318-368.
26. Barry KJ, Gogjian MA, Stein BM: Small animal model for investigation of subarachnoid haemorrhage and cerebral vasospasm. *Stroke* 10:538-541, 1979
27. Ohta T, Kajikawa H, Funatsu N, Yoshikawa Y, Someda K: Cerebral vasospasm and its relaxation responses to vasodilators: Pathological study of severe prolonged vasospasm. In: *Cerebral Arterial Spasm. Proceedings of the 2nd International Workshop*, Amsterdam, Wilkins RH (ed), Baltimore, Williams and Wilkins, 1980 pp. 132-138
28. Espinosa F, Weir B, Boisvert D, Overton T, Castor W: Chronic cerebral vasospasm after large subarachnoid hemorrhage in monkeys. *J Neurosurg* 57:224-232, 1982.
29. Peerless SJ, Fox AJ, Komatsu K, Hunter IG: Angiographic study of vasospasm following subarachnoid haemorrhage in monkeys. *Stroke* 13:473-479, 1982
30. Brawley BW, Strandness DE Jr, Kelly WA: The biphasic response of cerebral vasospasm in experimental subarachnoid haemorrhage. *J Neurosurg* 28:1-8, 1968
31. Kuwayama A, Zervas NT, Belson R, Shintani A, Pickren K: A model for experimental cerebral arterial spasm. *Stroke* 3:49-56, 1972
32. Nagai H, Noda S, Mabe H: Experimental cerebral vasospasm. Part 2: Effects of vasoactive drugs and sympathectomy on early and late spasm. *J Neurosurg* 42:420-428, 1975
33. Kågstöm E, Nilsson PE, Svendgaard NA: Clinical and experimental spasm of the cerebral vessels. *Excerpta Med Int Cong Series* 193:60, 1969
34. Simeone FA, Trepper PJ, Brown DJ: Cerebral blood flow evaluation of prolonged experimental vasospasm. *J Neurosurg* 37:302-311, 1972
35. Frazee JG: A primate model of chronic cerebral vasospasm. *Stroke* 5:612-614, 1982
36. Fisher CM, Kistler JP, Davis JM: Relation of cerebral vasospasm to subarachnoid haemorrhage visualized by computerized tomographic scanning. *Neurosurgery* 6:1-9, 1980
37. Fraser RAR, Stein BM, Barrett RE, Pool JL: Noradrenergic mediation of experimental cerebrovascular spasm. *Stroke* 1:356-362, 1970
38. Lobato RD, Mariñ J, Salas M, Burgos J, Rivilla F, Garcia AG: Effect of experimental subarachnoid hemorrhage on the adrenergic innervation of cerebral arteries. *J Neurosurg* 53: 477-479, 1980
39. Fein JM: Cerebral energy metabolism after subarachnoid hemorrhage. *Stroke* 6:1-18, 1975.

40. Boullin DJ, Aitken V, du Boulay GH, Tagari P: The calibre of cerebral arteries of the rat studied by carotid angiography: A model system for studying the aetiology of human cerebral arterial constriction after aneurysmal rupture. *Neuroradiology* 21:245-252, 1981.

Subarachnoid haemorrhage in the rat: Cerebral blood flow and glucose metabolism during the late phase of cerebral vasospasm

Tia J Delgado, Mohamed A-R Arbab,*Nils H Diemer and Niels-Aage Svendgaard

*From the Neurosurgical Research Department, University Hospital, Lund, Sweden and the *Neuropathological Institute, University of Copenhagen, Denmark*

SUMMARY

A double isotope technique for simultaneous measurement of cerebral blood flow (CBF) and glucose metabolism (CMRglu) was applied to a subarachnoid haemorrhage (SAH) model in the rat. Cisternal injection of 0.07 ml blood caused a rather uniform 20% reduction in cerebral blood flow together with an increase in glucose utilization of 30% during the late phase of vasospasm. In one third of the SAH animals, there were focal areas where the flow was lowered to 30% of the control values, and the glucose uptake increased to about 250% of control. We suggest that blood in the subarachnoid space via a neural mechanism induces the global flow and metabolic changes, and that the foci are caused by vasospasm superimposed on the global flow and metabolic changes.

In the double isotope autoradiographic technique, ^{14}C -iodoantipyrine and ^3H -deoxyglucose were used for CBF and CMRgl measurements respectively, in the same animal. In half of the sections, the ^{14}C -iodoantipyrine was extracted using 2,2 dimethoxypropane before the section was placed on a ^3H and ^{14}C sensitive film. The other sections were placed on X-ray film with an emulsion insensitive to ^3H . The validity of the double isotope method was tested by comparing the data with the data obtained in animals receiving a single isotope. The CBF and metabolic values obtained in the two groups were similar.

INTRODUCTION

Haemorrhage into the subarachnoid space from a ruptured intracranial aneurysm can induce arterial vasospasm and a reduction in cerebral blood flow (CBF) (Heros et al. 1976; Wilkins 1977). The relationship between vasospasm and CBF has not been clarified. CBF studies using the ^{133}Xe clearance method during the late phase of vasospasm in patients have not found a good correlation between the global CBF reduction commonly seen after a subarachnoid haemorrhage (SAH) and vasospasm. In contrast, regional CBF reductions have been described in areas supplied by arteries in spasm (Ferguson et al. 1972; Heilbrun et al. 1972; Bergvall et al. 1973; Ishii 1979). Compared to the large number of clinical studies examining CBF post SAH, only a few investigators have studied cerebral metabolism after a SAH (Grubbe et al. 1977; Martin et al.

1984; Powers et al. 1985). These investigators found a reduced oxygen metabolism (CMRO_2) post SAH.

Several experimental models have reproduced the late vasospasm and CBF changes seen in patients with a SAH (Hashi et al. 1972; Simeone et al. 1972; Chan et al. 1984; Sahlin et al. 1986). A few investigations have combined CBF studies with measurements of cerebral metabolism (Hashi et al. 1972; Boisvert et al. 1979; Sahlin et al. 1986). Only the latter authors found a decrease in CBF and CMRO_2 post SAH. Two studies evaluating CBF or metabolism post SAH have been performed in the rat. Solomon et al. (1984) measured hemispheric CBF in the acute stage using a microsphere technique, and Fein (1975) did regional metabolic studies at selected time points during the first twelve hours after a SAH.

The autoradiographic techniques developed by Reivich et al. (1969), Sakurada et al. (1978) and Sokoloff et al. (1977) allow evaluation of local CBF or local glucose metabolism in animals. The application of the autoradiographic technique to a SAH model would provide information about CBF and metabolism and their interrelationship that cannot be obtained with conventional techniques. In a previous communication, we presented an angiographical SAH model in the rat where it was demonstrated that cisternal blood injection produces a biphasic pattern of vasospasm with a maximal acute spasm at ten minutes and a maximal late spasm at two days (Delgado et al. 1985). In the present study, we describe a double isotope autoradiographic technique and apply it to evaluate local CBF and cerebral glucose metabolism (CMRglu) in the late phase of vasospasm in the rat.

METHOD AND MATERIAL

The experiments were performed on male Sprague-Dawley rats (SPF strain, Møllegaards Avlslaboratorium, Køge, Denmark) weighing between 280 and 400g. The animals were fasted for 12–18 hours before the CBF and/or CMRglu studies.

EXPERIMENTAL SAH MODEL

Under ether anaesthesia, homologous blood (0.07 ml) was injected via a previously implanted subcutaneous catheter connected to

the cisterna magna, as described previously (Delgado et al. 1985). The catheter was implanted three to seven days before the blood injection. Control animals received saline via the catheter. Two days after the intracisternal injection, the CBF and CMRglu studies were performed.

AUTORADIOGRAPHIC LOCAL CBF MEASUREMENTS

Local CBF was measured according to the technique described by Sakurada et al. (1978) as modified by Gjedde et al. (1980). Anaesthesia was initiated with 4% halothane. The animals were intubated and artificially ventilated with a 70% nitrous oxide and a 30% oxygen mixture. During the surgical procedure, 0.75% halothane was added to the gas mixture. Both femoral arteries were cannulated for blood pressure monitoring and for blood sampling. A catheter was inserted into one femoral vein for injection of Heparin (Vitrum, 75 IU/kg), Celocurin (suxamethonium chloride, Vitrum, 3 mg/kg) and for later infusion of the tracer. The CBF study was carried out not before 30 minutes after the halothane was switched off. The mean arterial blood pressure (MABP) was continuously monitored and blood gases were controlled. The temperature was kept around 37°C. The haematocrit was measured.

One arterial catheter was connected to a constant velocity withdrawal pump for mechanical integration of tracer concentration as described by Gjedde et al. (1980). Simultaneously 60 μ Ci of ^{14}C -iodoantipyrine (Amersham) was given as an i.v. bolus. Arterial blood (122 μ l) was withdrawn over 20 seconds. Immediately after, the animal was decapitated and the brain removed and immersed in isopentane chilled to -50°C .

The arterial blood sample was transferred to counting vials containing 0.75 ml of a Soluene (Packard) and isopropanol mixture (1:1). Hydrogen peroxide (0.3 ml, 30% solution) was added to the vials. After storing the vials for four to six hours at room temperature, 7.5 ml of an Instagel (Packard) and 0.5 N HCL mixture (9:1) were added, and the samples were maintained at $+4^\circ\text{C}$ for about 12 hours before beta scintillation counting with a program that included quench correction (Hewlett Packard).

The ^{14}C activity in the tissue was determined after sectioning the brain in 20 μm sections at -20°C . The sections were dried on a hot

plate (+60°C) and then placed on an X-ray film (Kodak MIN-R) together with eight ^{14}C methylmetacrylate standards (Amersham). The exposure time was 16 days.

Autoradiograms were analyzed using the memory of a Leitz TAS plus (R) image analyzer. Brain regions were encircled using a light pen and the average optical density obtained. The computer converted the optical density to ^{14}C activity using a third degree polynomial curve determined from the calibrated standards as described by Reivich et al. (1969).

The CBF was calculated from the brain tissue ^{14}C activity determined by autoradiography using the equation of Gjedde et al. (1980):

$$f^{bl} = \frac{C_{Br}(T)}{E(T) \int_0^T C_a(t) dt}$$

where f^{bl} is the blood flow per unit mass, $C_{Br}(T)$ the isotope content, $E(T)$ the net extraction fraction of the isotope in the time from $t=0$ to $t=T$, t = the variable time and T = the experimental time (20 seconds). $C_a(t)$ is the arterial blood concentration of the isotope at time t .

AUTORADIOGRAPHIC LOCAL CMRglu MEASUREMENTS

The local CMRglu was determined by the method described by Sokoloff et al. (1977). The anaesthesia and cannulation procedures were as described. 50 μCi ^{14}C -deoxyglucose (Amersham) was infused i.v. over 30 seconds. Fifteen samples of arterial blood (100 μl) were taken during a 45 minute period. The blood samples were immediately centrifuged and the plasma was separated for determination of ^{14}C -deoxyglucose by beta scintillation counting and for determination of glucose concentration using a glucose oxidase method (Greiner G 300). After the last blood sample, the animal was decapitated and the brain frozen as described, for later sectioning. The plasma samples (40 μl) for measurement of ^{14}C -deoxyglucose activity were placed in vials containing 0.75 ml of a Soluene and isopropanol mixture (1:1). Instagel with HCL was added before scintillation counting as previously described.

The ^{14}C activity in the tissue was determined after cutting the brain in 20 μm sections. The sections were processed for autoradi-

ography using X-ray film (Kodak, MIN-R) and 16 days exposure time. The density of the autoradiograms was measured using a Leitz TAS plus image analyzer as described previously and converted to ^{14}C activity using a curve determined from eight ^{14}C methylmethacrylate standards (Amersham). The local CMRglu was calculated from the plasma deoxyglucose integral, the plasma glucose concentration and the ^{14}C -activity in the brain tissue according to Sokoloff et al. (1977). The values used for the lumped constant and the rate constants for the normal animals were those determined by Sokoloff et al. (1977).

SIMULTANEOUS LOCAL CBF AND CMRglu MEASUREMENTS

Autoradiographic determination of local CBF and CMRglu was made in the same animal using ^{14}C -iodoantipyrine and ^3H -deoxyglucose, respectively. Anaesthesia and surgical procedures were as described. The animal was injected i.v. with 750 uCi ^3H -deoxyglucose (Amersham) and frequent blood samples were taken over a period of 45 minutes for the determination of the plasma deoxyglucose integral. After the last sample, one of the arterial catheters was connected to a constant velocity withdrawal pump and 60 uCi of ^{14}C iodoantipyrine was given as an i.v. bolus. Arterial blood (122 ul) was withdrawn over 20 seconds. The animal was decapitated and the brain frozen. The concentration of radioactivity in the plasma and blood samples was measured using a beta-liquid scintillation counter with programs for quench correction and double label counting (of the blood samples).

Brain radioactivity content was measured using autoradiography of 20 μm sections. Sections for detection of ^3H -deoxyglucose content were washed immediately after cutting, 2x1 minute in 2,2 dime-thoxypropane (DMP) which solubilized the iodoantipyrine completely without removing deoxyglucose (Diemer and Rosenørn 1981; Gjedde and Diemer 1985; Owman and Diemer 1985). These sections were placed on ^3H sensitive film (LKB Ultrofilm). The exposure time was 19 days. The neighboring sections were placed on an X-ray film (Kodak MIN-R) with a protective gelatin coating which absorbed the ^3H -radiation without affecting the ^{14}C -radiation. No penetration of the coating by the ^3H -radiation was found with this exposure time even when the amount of ^3H -deoxyglucose injected

was increased three times (to be published). Two sets of standards were used; one calibrated for brain tissue ^{14}C content, and one for ^3H content in DMP treated sections. The optical density of the same brain areas on the two autoradiograms was measured using the Leitz TAS Plus image analyzer and converted to ^{14}C or ^3H activity.

ESTIMATION OF THE LUMPED CONSTANT.

The lumped constant (LC) which is the correction factor due to the use of deoxyglucose instead of the native substrate glucose (Sokoloff et al. 1977), was calculated for the SAH animals. The LC is dependent mainly on brain glucose content (Crane et al. 1981; Gjedde 1982). The brain glucose content can be determined by the use of the nonmetabolizable glucose analogue, 3-0-methylglucose, since the apparent distribution volume of this tracer only depends on the glucose content. Given the kinetic constants of glucose transport, and the apparent volume of distribution, i.e. the activity in brain tissue divided by the activity in plasma at equilibrium, the brain glucose content (Me) can be calculated (Diemer and Gjedde 1983; Gjedde and Diemer 1983):

$$\text{Me} = V_e^M (\infty) \left[K_t + C_a \right] - K_e$$

V_e^M = virtual steady state distribution volume for 3-0-methylglucose, K_+ = Michaelis constant, C_a = arterial plasma concentration of glucose and K_e = half saturation content of brain glucose.

Having calculated the brain glucose content, the LC can be determined:

$$\text{LC} = \frac{K_3^D}{K_3^G} \left[\frac{V_e^M (\infty) C_a}{\text{Me}} \right]$$

K_3^D and K_3^G are the rate constants for phosphorylation of deoxyglucose and glucose, respectively.

In the animals used to determine the LC, anaesthesia and cannulation procedures were as described. At time zero, the animals received 30 uCi ^{14}C -deoxyglucose and 600 uCi ^3H -3-0 methylglucose as an i.v. bolus injection. Blood samples were taken repeatedly during ten minutes for the determination of the plasma deoxyglucose,

glucose and 3-0-methylglucose concentrations. Immediately after the last blood sample, the animal was decapitated and the brain frozen. The double autoradiograms were made from adjacent 20 μ m sections.

One set of the sections was exposed to a highly sensitive film (LKB Ultrofilm) that reflected both isotopes. The other set was exposed to an X-ray film coated with a protective gelatine layer which only reflected the ^{14}C isotope. Based on the plasma and the brain tissue concentration of 3-0-methylglucose, the LC was calculated.

EXPERIMENTAL DESIGN

There were six experimental groups.

Group I Four animals with local CBF measurements using ^{14}C -iodoantipyrine.

Group II Four animals with local CMRglu measurements using ^{14}C -deoxyglucose.

Group III Four animals with simultaneous CBF and CMRglu measurements using ^{14}C -iodoantipyrine and ^3H -deoxyglucose.

Group IV Four animals with simultaneous CBF and CMRglu measurements two days after cisternal injection of 0.07 ml saline.

Group V Three animals for evaluation of the LC two days after cisternal blood injection using ^{14}C -deoxyglucose and ^3H -3-0 methylglucose.

Group VI Eight animals with simultaneous CBF and CMRglu measurements two days after cisternal injection of 0.07 ml blood.

STATISTICS

The data were analyzed with the student t-test for two group comparisons and with the Bonferroni t-test for multiple comparisons.

RESULTS

The physiological parameters for the experimental groups are shown in Table I. All animals had rectal temperatures close to 37°C. The PaO_2 was about 130 mmHg. The PaCO_2 was about 37 mmHg. The pH was close to 7.4. There were no significant differences in the MABP between the experimental groups.

LCBF AND LCMRglu IN NORMAL ANIMALS USING A SINGLE OR DOUBLE TRACER TECHNIQUE.

The values of LCBF and LCMRglu obtained in normal anaesthetized animals with single or double isotope autoradiography are given in Table II. There was no significant difference in CBF and CMRglu between the single and double isotope groups. Also, the values are comparable to those reported by others for anaesthetized rats (Abdul-Rahman et al. 1980; Tamura et al. 1981; Ingvar and Siesjö 1982; Iadecola et al. 1983). The double isotope autoradiography revealed a good correlation between LCBF and LCMRglu. The correlation was illustrated by plotting the flow values as a function of glucose metabolism (Fig 1A). The linear regression line was calculated and found to be: $\text{LCBF} = 1.90 \times \text{LCMRglu} + 42.0$. The correlation coefficient ($r = 0.75$) was comparable to the coefficients reported by others for anaesthetized rats (Sokoloff 1978; Nakai et al. 1983).

LCBF AND LCMRglu IN ANIMALS TWO DAYS AFTER INTRACISTERNAL INJECTION OF SALINE OR BLOOD.

The LCBF and LCMRglu values in the animals receiving cisternal saline injection were similar to those obtained in normal anaesthetized animals (Fig 1A and Table II and III). The regression line calculated for saline injected animals was $\text{LCBF} = 2.35 \times \text{LCMRglu} + 19.3$ with a correlation coefficient of 0.70. The values for the normal and saline injected animals were pooled for comparison with the SAH animals. The regression line calculated for the pooled animals was $\text{LCBF} = 2.16 \times \text{LCMRglu} + 29.8$ with a correlation coefficient of 0.73 (Fig 1B).

Table 1
Physiological parameters in the experimental groups

experimental groups	MABP(mmHg)	Hct	glucose(mmol/l)	temp °C	ph	PaO ₂ (mmHg)	PaCO ₂ (mmHg)	n
1. LCBF in normal animals	117±5	43±1	-	37.1±0.1	7.38±0.01	138±9	37.8±1.4	4
2. LCMRGlu in normal animals	123±10	46±1	10.3±2.7	37.3±0.2	7.40±0.04	159±6	36.9±0.4	4
3. LCBF and LCMRGlu in normal animals	120±4	45±1	11.1±1.5	37.0±0.1		157±5	36.9±1.2	4
4. LCBF and LCMRGlu 2D after cisternal saline injection	116±8	43±1	9.7±0.7	37.0±0.01	7.37±0.02	127±9	37.5±1.1	4
5. Evaluation of lumped constant 2D after cisternal blood injection	114±9	45±2	11.3±0.2	37.2±0.2	7.40±0.01	129±3	36.7±1.5	3
6. LCBF and LCMRGlu 2D after cisternal blood injection	112±5	45±1	13.1±1.2	37.2±0.1	7.41±0.02	123±4	36.4±1.3	8

Values are means ± S.E.M.

MABP = mean arterial blood pressure

Table II

Local cerebral blood flow (LCBF) and glucose metabolism (LCMRglu) in anaesthetized, paralyzed rats using a single or double isotope technique

Region	LCBF(ml/min x 100 g)		LCMRglu(umol/min x 100g)	
	single isotope	double isotope	single isotope	double isotope
frontal cortex	144+4	151+4	56+2	64+4
septum	106+13	116+12	46+2	48+5
hypothalamus	104+10	114+5	41+5	38+3
corpus callosum	57+2	51+4	21+2	22+5
caudate putamen	154+15	160+4	72+6	70+4
sensorimotor cortex	150+16	154+15	61+2	67+3
parietal cortex	135+8	139+12	59+3	58+3
hippocampus	94+5	96+6	51+2	45+3
thalamus	174+14	173+7	68+6	62+3
occipital cortex	123+11	138+15	58+7	56+5
superior colliculus	142+6	146+8	55+4	56+1
inferior colliculus	183+14	184+3	57+6	64+5
cerebellar cortex	93+11	96+11	37+5	36+2
dentate nucleus	138+25	154+17	47+5	46+7
vestibular nucleus	138+10	159+12	54+6	49+2
cochlear nucleus	153+14	152+7	48+8	47+3
trigeminal nucleus	154+17	150+3	41+7	42+3
facial nucleus	170+12	154+15	39+3	39+4
	N=4	N=4	N=4	N=4

Values are means $\pm$ S.E.M.

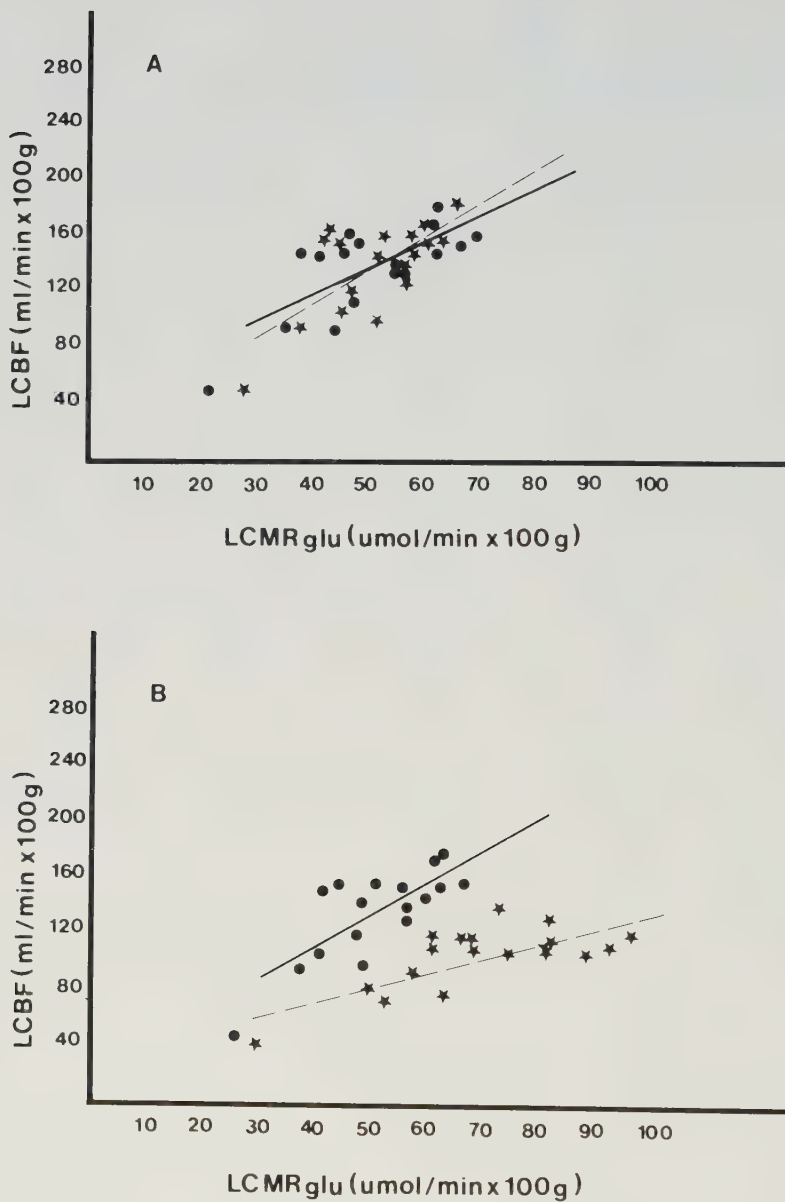


Fig 1: LCBF plotted as a function of LCMRglu in 18 brain regions. A. Normal animals (solid line and dots) versus animals injected intracisternally with saline (dashed line and stars). B. Pooled values from four normal animals and four animals with cisternal saline injection (solid line and dots) compared to animals with cisternal blood injection (dashed line and stars). All animals were anaesthetized and paralyzed.

Table III

Local cerebral blood flow (LCBF) and glucose metabolism (LCMRglu) in anaesthetized, paralyzed rats two days after cisternal saline injection

Region	LCBF(ml/min x 100g)	LCMRglu(umol/min x 100g)
frontal cortex	150 $\pm$ 4	57 $\pm$ 2
septum	120 $\pm$ 5	47 $\pm$ 3
hypothalamus	107 $\pm$ 4	46 $\pm$ 3
corpus callosum	56 $\pm$ 3	28 $\pm$ 1
caudate putamen	160 $\pm$ 8	64 $\pm$ 4
sensorimotor cortex	161 $\pm$ 4	59 $\pm$ 3
parietal cortex	143 $\pm$ 5	57 $\pm$ 3
hippocampus	100 $\pm$ 5	53 $\pm$ 4
thalamus	180 $\pm$ 7	66 $\pm$ 7
occipital cortex	133 $\pm$ 6	58 $\pm$ 3
superior colliculus	159 $\pm$ 5	57 $\pm$ 4
inferior colliculus	169 $\pm$ 15	61 $\pm$ 4
cerebellar cortex	97 $\pm$ 5	38 $\pm$ 1
dentate nucleus	146 $\pm$ 17	52 $\pm$ 1
vestibular nucleus	168 $\pm$ 17	54 $\pm$ 6
cochlear nucleus	168 $\pm$ 9	44 $\pm$ 1
trigeminal nucleus	160 $\pm$ 9	43 $\pm$ 2
facial nucleus	157 $\pm$ 8	46 $\pm$ 3
	N=4	N=4

Values are means $\pm$ S.E.M.

There were two types of changes in LCBF and LCMRglu seen two days after cisternal blood injection. All animals showed a generalized or global decrease in CBF and an increased uptake of deoxyglucose. Three of the eight (38%) SAH animals, also had foci with markedly decreased flow and increased deoxyglucose uptake (Fig 2A and B).

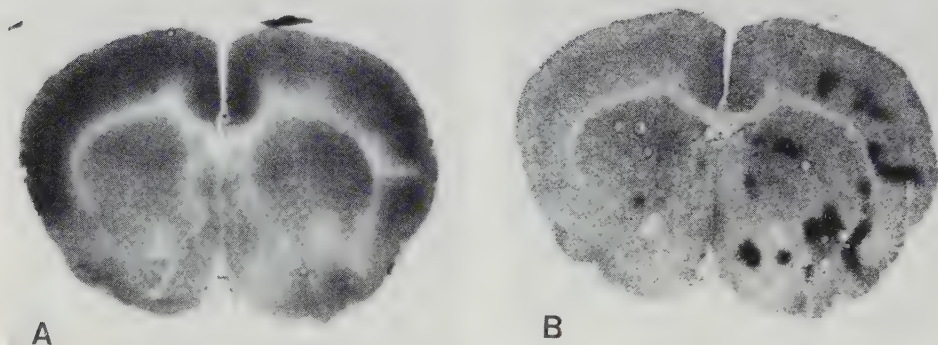


Fig 2: A. ¹⁴C-iodoantipyrine autoradiogram at the level of caudate-putamen showing low flow areas in a rat brain two days after a SAH. B. Adjacent section of a ³H-deoxyglucose autoradiogram demonstrating the corresponding areas with increased deoxyglucose uptake.

The LC used for calculating the CMRglu in the focal areas was 0.61, which reflects the effect of oligemia (Ginsberg and Reivich 1979). The standard deviation was 0.03. It was calculated from three SAH animals receiving 3-O-methylglucose and ¹⁴C-deoxyglucose. One of the animals had multiple foci. In the areas outside the foci, the LC was 0.48 ± 0.07 (mean $\pm$ SD), which is similar to the value calculated by Sokoloff et al. (1977) for normal rats (0.483 ± 0.107).

The SAH animals had a quite uniform reduction in the mean CBF of about 20% as compared to the pooled normal and saline injected animals (Table IV). The individual variations in CBF were greater in the SAH animals than in the control animals. The CBF reduction was significant ($p < 0.05 - p < 0.01$) in all the regions examined but the dentate nucleus. The reduction in CBF in regions supplied by the vertebro-basilar arteries was similar to that seen in regions supplied by the internal carotid arteries.

In contrast, there was an increase in CMRglu in the SAH animals (Table IV). The individual variations in CMRglu were greater in the SAH animals than in the controls. The increase in deoxyglucose uptake was about 30% in the brain regions examined, except for the cochlear and the vestibular nuclei where the increase was close to 100%. The increase in deoxyglucose was significant ($p < 0.05$ – $p < 0.001$) in 12 of 18 regions. Apart from the cochlear and vestibular nuclei, regions supplied by the vertebro-basilar arteries showed similar increases in CMRglu as the regions supplied by the internal carotid artery.

The relationship between LCBF and LCMRglu was different in SAH animals as compared to control animals. This was reflected in the regression lines for SAH and control animals, respectively (Fig 1B). The regression line calculated for the SAH animals was $LCBF = 1.04 \text{ LCMRglu} + 35.7$ with a correlation coefficient of 0.72.

In addition to the global alterations in flow and metabolism, there also were focal changes in CBF and CMRglu. The deoxyglucose uptake in the foci was increased by about 250% (Table V). In the corresponding areas, the flow was decreased to about 30% of control (Table V). In the animals with foci, the global changes in flow and metabolism were somewhat greater than those in animals without foci. The foci were seen in all three animals in the frontal, sensorimotor and parietal cortex, in the caudate-putamen and thalamus. The size of the focal changes varied greatly. On coronal sections, the extension of the foci varied from a few tenths of a millimeter to 1.5 mm. By counting the number of sections showing the same focal change, it could be estimated that the anterior-posterior extension was of the same magnitude. There was a rather narrow zone of transition between the focal changes and the surrounding parenchyma without perifocal increases in flow.

All the SAH animals were drowsy and exhibited minor weight loss. None of the animals had detectable focal neurological deficits.

Table IV

Local cerebral blood flow (LCBF) and glucose metabolism (LCMRglu) in anaesthetized, paralyzed rats two days after cisternal injection of 0.07 ml blood

	LCBF(ml/min x 100g)	LCMRglu(umol/min x 100g)
Regions supplied by the internal carotid arteries		
frontal cortex	111 $\pm$ 12 (74%)**	81 $\pm$ 4 (135%***
septum	101 $\pm$ 12 (81%)*	58 $\pm$ 6 (123%)n.s.
hypothalamus	86 $\pm$ 8 (78%)**	50 $\pm$ 4 (122%)n.s.
corpus callosum	43 $\pm$ 3 (81%)**	30 $\pm$ 8 (115%)n.s.
caudate putamen	117 $\pm$ 11 (73%)**	93 $\pm$ 7 (139%)**
sensorimotor cortex	116 $\pm$ 16 (73%)*	82 $\pm$ 5 (130%)**
parietal cortex	114 $\pm$ 14 (81%)*	75 $\pm$ 6 (132%)**
hippocampus	79 $\pm$ 8 (81%)*	64 $\pm$ 7 (131%)*
thalamus	135 $\pm$ 18 (77%)*	82 $\pm$ 5 (128%)**
Regions supplied by the vertebro-basilar arteries		
occipital cortex	109 $\pm$ 11 (81%)*	69 $\pm$ 10 (121%)n.s.
superior colliculus	124 $\pm$ 13 (81%)*	67 $\pm$ 6 (118%)n.s.
inferior colliculus	141 $\pm$ 16 (80%)*	73 $\pm$ 8 (118%)n.s.
cerebellar cortex	75 $\pm$ 6 (78%)*	53 $\pm$ 8 (143%)*
dentate nucleus	121 $\pm$ 16 (82%)n.s.	68 $\pm$ 6 (139%)**
vestibular nucleus	125 $\pm$ 16 (77%)*	96 $\pm$ 8 (188%***
cochlear nucleus	109 $\pm$ 12 (68%)**	88 $\pm$ 14 (196%)**
trigeminal nucleus	120 $\pm$ 13 (77%)*	61 $\pm$ 7 (145%)*
facial nucleus	121 $\pm$ 12 (78%)*	61 $\pm$ 7 (145%)*
	N=8	N=8

Values are means $\pm$ S.E.M.

(%) of control (pooled normal and saline injected animals)

*p<0.05, **p<0.01, ***p<0.001

Table V

Cerebral blood flow and glucose metabolism in the foci noted in three rats two days after SAH

Region	CBF ml/min x 100g	% control	CMRglu umol/min x 100g	% control	N of animals
frontal cortex	43 $\pm$ 15	29%	115 $\pm$ 15	192%	3
sensorimotor cortex	47 $\pm$ 7	30%	131 $\pm$ 24	208%	3
parietal cortex	49 $\pm$ 4	34%	134 $\pm$ 9	235%	3
occipital cortex	46 $\pm$ 21	27%	126 $\pm$ 4	233%	2
cerebellar cortex	23 $\pm$ 15	25%	110 $\pm$ 21	290%	1
caudate putamen	57 $\pm$ 14	37%	174 $\pm$ 6	252%	3
thalamus	62 $\pm$ 6	35%	148 $\pm$ 43	231%	3
hypothalamus	39	35%	82	195%	1
hippocampus	53 $\pm$ 4	54%	116 $\pm$ 7	237%	1

Values are means $\pm$ S.E.M.

DISCUSSION

The present study demonstrates that the LCBF and LCMRglu values obtained with double isotope autoradiography using ^{14}C -iodoantipyrine and ^3H -deoxyglucose respectively, are comparable to those obtained with single isotope autoradiography using ^{14}C -iodoantipyrine and ^{14}C -deoxyglucose.

Recently, several communications describing double isotope techniques for measurement of LCBF and LCMRglu have been published. Short lived gamma emitting isotopes and the beta emitting ^{14}C were used by Jones et al. (1979), Blasberg et al. (1981) and Mies et al. (1981) to measure flow and glucose metabolism, respectively. Sako et al. (1984) used the short lived positron emitting nucleotide ^{18}F for evaluation of glucose metabolism and ^{14}C for flow measurement. Furlow et al. (1983) used ^{14}C -iodoantipyrine for flow measurements and ^{14}C -deoxyglucose for evaluation of glucose metabol-

ism. The extraction with chloroform used by these authors, besides removing ^{14}C -iodoantipyrine, also extracts some of the ^3H -deoxyglucose (Jones and Greenberg 1985).

There are several advantages in using ^3H -deoxyglucose and ^{14}C -iodoantipyrine in the double isotope technique. The autoradiographic resolution is high using tritiated deoxyglucose and the same standards can be used from experiment to experiment. A relatively short time period is required for exposure of the films. In addition, the isotopes are commercially available and the hazards of working with gamma emitting isotopes are avoided. On the other hand, it has been suggested (Sako et al. 1984) that part of the ^3H -deoxyglucose-phosphate or free deoxyglucose might be lost during the extraction of iodoantipyrine. However, it has been found that DMP does not influence the content or distribution of deoxyglucose (Diemer and Rosenørn 1981). It has also been proposed that there is a different degree of self-absorption of the ^3H radiation in white and grey matter (Alexander et al. 1981) which could lead to an underestimation of the CMRglu in white matter.

The data presented in this study demonstrate that there are both global and focal changes in CBF and glucose metabolism during the late phase of vasospasm after a SAH. The global decrease in CBF after cisternal injection of 0.07 ml blood was approximately 20% while the increase in deoxyglucose uptake was about 30% except in the cochlear and vestibular nuclei where it was about three times greater. In the foci, the flow was decreased to about 30% and the glucose uptake increased to about 250% of control.

A decrease in cerebral blood flow has been noted in experimental SAH in the acute phase in monkeys (Hashi et al. 1972; Petruk et al. 1972; Kamiya et al. 1983); rabbits (Logothetis et al. 1983; Umansky et al. 1983) and rats (Solomon et al. 1985) and in the late phase of vasospasm in monkeys (Simeone et al. 1972; Sahlin et al. 1986) and rabbits (Logothetis et al. 1983). CBF reductions have also been described in patients following a SAH (James et al. 1968; Zingesser et al. 1968; Heilbrun et al. 1972; Bergvall et al. 1973; Ishii 1979; Meyer et al. 1983; Mickey et al. 1984). In clinical investigations and in several experimental studies, (Hashi et al. 1972; Petruk et al. 1972; Simeone et al. 1972; Sahlin et al. 1986) the global CBF reduction was found to be poorly correlated to the degree of angiographical vasospasm. Basically there was a global reduction in CBF irrespective

of the presence or absence of a generalized spasm. Further evidence supporting the poor correlation between CBF reduction and angiographical vasospasm is provided by a comparison of the CBF studies of Solomon et al. (1985) with our angiographical data (Delgado et al. 1985). Solomon et al. (1985) found a progressive decrease in CBF during the first 60 minutes following cisternal injection of 0.3 ml blood in rats, while our angiographical studies have shown an inverse relationship for spasm; from a maximal spasm of 39% at ten minutes, it progressively decreased to reach a degree of spasm of 15% at 60 minutes after the SAH (Delgado et al. 1985). Additional evidence is that a change in the amount of cisternal blood from 0.07 to 0.3 ml increased the CBF reduction from 20 to 30% (unpublished observation) although the larger amount of blood only increased the degree of spasm from 24 to 27% at two days post SAH (Delgado et al. 1985). One explanation for the discrepancy between flow and the degree of vasospasm could be, that the CBF reduction is secondary to a change in cerebral metabolism. Another possibility is that the flow reduction is secondary to spasm in the small not angiographically visible conducting arteries and that a larger amount of blood produces a greater spasm in these vessels by penetrating deeper into the perivascular spaces. It is less likely that the arterioles or resistance vessels are in spasm since there is an increase in cerebral blood volume, probably caused by a dilatation of the resistance vessels (Grubb et al. 1977; Martin et al. 1984).

There was a global increase in deoxyglucose uptake in the SAH animals of about 30% which probably represents an anaerobic glycolysis. That it is an anaerobic glycolysis is strongly supported by the decrease in the cerebral metabolic rate of oxygen (CMRO_2) described by Grubb et al. (1977), Martin et al. (1984) and Voldby et al. (1985) in SAH patients and by Sahlin et al. (1986) in monkeys during the late phase of spasm. Further evidence for an anaerobic glycolysis is provided by studies showing an increased lactate/ pyruvate ratio in the cerebrospinal fluid in SAH patients (Kågström et al. 1973; Fujishima et al. 1975; Pasqualin et al. 1985). An increased lactate/ pyruvate ratio has been found in the acute phase after an experimental SAH in dogs (Shannon et al. 1972; Sugi et al. 1975) and in monkeys (Fein 1975). Fein (1975) also described an increased lactate/ pyruvate ratio in brain tissue obtained from rats in the acute phase after a SAH. The anaerobic glycolysis can hardly be explained on the basis

of reduced CBF. It has been demonstrated by Shannon et al. (1970), Bruce et al. (1972) and Eklöf and Siesjö (1972) that the cerebral metabolism does not change in an anaerobic direction until the CBF is reduced by 50% or more. Thus, in normal animals a CBF reduction of up to 50% will be adequately balanced by an increased oxygen extraction, while in SAH animals there seems to be a reduction in CMRO_2 , probably secondary to a decrease in the extraction rate of oxygen as suggested by Fein (1975). Fein found an immediate decrease in CMRO_2 in monkeys following an acute SAH despite an initially preserved blood flow. The decrease in CMRO_2 was maintained although the oxygen supply was sufficient. Supporting clinical evidence was presented by Voldby et al. (1985) who found that in SAH patients, there was a decrease in the arteriovenous difference of oxygen not correlated to the reduction in flow and also that the CMRO_2 reduction was greater than the decrease in CBF.

The changes in CBF and metabolism cannot be explained simply by an increase in intracranial pressure. Fein (1975) found that the changes occurred irrespective of whether or not blood was injected under isobaric or hyperbaric conditions. Sahlin et al. (1986) showed that the changes were not correlated to the level of sagittal sinus pressure. Finally, the small amount of blood injected in this study when combined with aspiration of cerebrospinal fluid, probably only affects the intracranial pressure minimally, suggesting that the changes are caused by the presence of blood in the subarachnoid space.

Focal low flow areas with high glucose uptake appeared predominantly in the cortex, caudate putamen and thalamus, and were seen in 1/3 of the SAH animals. The occurrence of focal changes has not been previously described in experimental SAH and the mechanism underlying their development is not clear. We suggest that foci develop due to a severe local spasm superimposed on the global flow reduction, i.e. spasm in long narrow arteries causes a substantial decrease in perfusion pressure in areas without adequate collateral circulation. It is interesting that Yamori et al. (1976) found that ischemic areas generally appeared in the same regions in stroke prone spontaneously hypertensive rats. These authors noted that a substantial part of the blood supply to these regions was via recurrent branches from the larger cerebral arteries. The combination of this angioarchitectural feature and spasm could lead to the markedly re-

duced flow in these areas. The foci could represent preinfarctions and correspond to the lucent areas seen with computerized tomography in SAH patients (Bryan et al. 1979; Saito et al. 1979).

How the changes in CBF, metabolism and cerebral vasospasm following a SAH are interrelated, and how blood in the subarachnoid space elicits these changes is not known. There is an increasing evidence supporting a poor correlation between vasospasm and the global component of the reduction in flow. Vasospasm could play a role in terms of eliciting focal changes which in turn could cause neurological deficits. Our data indicate that there is an altered setting in the CBF-metabolism relationship although there is still an interdependence between flow and metabolism following a SAH. Blood might alter the oxidative metabolic process and affect blood flow by a direct mechanism. However, given the uniform nature of the global changes in flow and metabolism a neural mechanism is more likely. In this context, it is interesting that stimulation of discrete areas in the dorsal medullary reticular formation and in the medial parabrachial nucleus has been shown to alter global cerebral metabolism with secondary effects on flow (Iadecola et al. 1983; Reis et al. 1985). Similarly, stimulation of discrete areas in the fastigial and in the lateral parabrachial nuclei has been shown to alter cerebral vascular tone (Miura and Reis 1969; Mraovitch et al. 1983).

In conclusion: The study has demonstrated that blood in the subarachnoid space induces a decrease in cerebral blood flow and an increase in glucose utilization consistent with an anaerobic glycolysis. We suggest that blood via a neural mechanism causes a global decrease in cerebral oxidative metabolism and flow, and that the focal changes are the result of vasospasm superimposed on preexisting global metabolic and flow changes.

ACKNOWLEDGEMENT

Supported from Einar Björkelund Foundation; Anna-Lisa and Sven-Eric Lundgren Foundation; Thorsten and Elsa Segerfalk Foundation; Danish Medical Research Council (12-4615); Medical Faculty, University of Lund; Swedish Medical Research Council (NO 04X-5300 and 14X-732).

REFERENCES

- Abdul Rahman A, Agardh CD, Siesjö BK (1980) Local cerebral blood flow in the rat during severe hypoglycemia, and in the recovery period following glucose injection. *Acta Physiol Scand* 109:307-314
- Alexander GM, Schwartzman RJ, Bell RD, Yu J, Renthal A (1981) Quantitative measurement of local cerebral metabolic rate for glucose utilizing tritiated 2-deoxyglucose. *Brain Res* 223:59-67
- Bergvall U, Steiner L, Forster DMC (1973) Early patterns of cerebral circulatory disturbances following subarachnoid haemorrhage. *Neuroradiology* 5:24-42
- Blasberg R, Molnar P, Groothuis D, Patlak C, Fenstermacher J (1981) Simultaneous measurement of blood flow and relative glucose utilization in ASV induced brain tumors. *J Cereb Blood Flow Metabol (Suppl 1)* 1:S68-S69
- Boisvert DPJ, Pickard JD, Graham DI, Fitch W (1979) Delayed effects of subarachnoid haemorrhage on cerebral metabolism and the cerebrovascular response to hypercapnia in the primate. *J Neurol Neurosurg Psychiatry* 42:892-899
- Bruce DA, Schutz H, Vapalahti M, Gunby N, Langfitt TW (1972) Interactions between cerebral blood flow and cerebral metabolism. *Surg Forum* 23:417-419
- Bryan RN, Shah CP, Hilal SC (1979) Evaluation of subarachnoid hemorrhage and cerebral vasospasm by computerized tomography. *CT* 3:144-152
- Chan RC, Durity FA, Thompson GB, Nugent RA, Kendall M (1984) The role of the prostacyclin thromboxane system in cerebral vasospasm following induced subarachnoid hemorrhage in the rabbit. *J Neurosurg* 61:1120-1128
- Crane PD, Pardridge WM, Braun LD, Nyerges AM, Oldendorf WH (1981) The interaction of transport and metabolism on brain glucose utilization: A reevaluation of the lumped constant. *J Neurochem* 36:1601-1604
- Delgado TJ, Brismar J, Svendgaard N Aa (1985) Subarachnoid haemorrhage in the rat: angiography and fluorescence microscopy of the major cerebral arteries. *Stroke* 16:595-602
- Diemer NH, Gjedde A (1983) Autoradiographic determination of brain glucose content and visualization of the regional lumped constant. *J Cereb Blood Flow Metabol* 3 (Suppl 1):S79-S80
- Diemer NH, Rosenørn J (1981) Determination of local cerebral blood flow and glucose metabolism or transfer by means of a double autoradiographic method. *J Cereb Blood Flow Metabol (Suppl 1)* 1:S72-S73
- Eklöf B, Siesjö BK (1972) The effect of bilateral carotid artery ligation upon the blood flow and the energy state of the rat brain. *Acta Physiol Scand* 86:155-165

- Espinosa F, Weir B, Overton T, Castor W, Grace M, Boisvert D (1984) A randomized placebo controlled double-blind trial of nimodipine after SAH in monkeys. *J Neurosurg* 60:1167-1175
- Fein JM (1975) Cerebral energy metabolism after subarachnoid hemorrhage. *Stroke* 6:1-8
- Ferguson GC, Harper AM, Fitch W, Rowan JO, Jennett B (1972) Cerebral blood flow measurements after spontaneous subarachnoid hemorrhage. *Europ Neurol* 8:15-22
- Fujishima M, Sugi T, Choki J, Yamaguchi T, Omae T (1975) Cerebrospinal fluid and arterial lactate, pyruvate and acid-base balance in patients with intracranial hemorrhages. *Stroke* 6:707-714
- Furlow TW, Martin RM, Harrison LEC (1983) Simultaneous measurement of local glucose utilization and blood flow in the rat brain: an autoradiographic method using two tracers labelled with carbon-14. *J Cereb Blood Flow Metabol* 3:62-66
- Ginsberg MD, Reivich M (1979) Use of the 2-deoxy-D-glucose method of local cerebral glucose utilization in the abnormal brain: Evaluation of the lumped constant during ischemia. *Acta Neurol Scand (Suppl)* 60 (72):226-227
- Gjedde A (1982) Calculation of cerebral glucose phosphorylation from brain uptake of glucose analogs in vivo: A re-examination. *Brain Res Rev* 4:237-274
- Gjedde A, Diemer NH (1983) Autoradiographic determination of regional brain glucose content. *J Cereb Blood Flow Metabol* 3:303-310
- Gjedde A, Diemer NH (1985) Double-tracer study of the fine regional blood-brain glucose transfer in the rat by computer-assisted autoradiography. *J Cereb Blood Flow Metabol* 5:282-289
- Gjedde A, Hansen AJ, Siemkowicz E (1980) Rapid simultaneous determination of regional blood flow and blood-brain glucose transfer in brain of rat. *Acta Physiol Scand* 108:321-330
- Grubb RL, Raichle ME, Eichling JO, Gado MH (1977) Effects of subarachnoid hemorrhage on cerebral blood volume, blood flow, and oxygen utilization in humans. *J Neurosurg* 46:446-453
- Hashi K, Meyer JS, Shinmaru S, Welch MA, Teraura T (1972) Cerebral hemodynamic and metabolic changes after experimental subarachnoid hemorrhage. *J Neurol Sci* 17:1-14
- Heilbrun MP, Olesen J, Lassen NA (1972) Regional cerebral blood flow studies in subarachnoid hemorrhage. *J Neurosurg* 37:36-44
- Heros RC, Zervas NT, Negoro M (1976) Cerebral vasospasm. *Surg Neurol* 5:354-362
- Iadecola C, Nakai M, Mraovitch S, Ruggiero DA, Tucker LW, Reis DJ (1983) Global increase in cerebral metabolism and blood flow produced by focal electrical stimulation of dorsal medullary reticular formation in rat. *Brain Res* 272:101-114
- Ingvar M, Siesjö BK (1982) Effect of nitrous oxide on local cerebral glucose utilization in rats. *J Cereb Blood Flow Metabol* 2:481-486
- Ishii R (1979) Regional cerebral blood flow in patients with ruptured intracranial aneurysms. *J Neurosurg* 50:587-594

- Jakubowski J, Bell BA, Symon L, Zawirski MB, Francis DM (1982) A primate model of subarachnoid hemorrhage: change in regional cerebral blood flow, autoregulation, carbon dioxide reactivity, and central conduction time. *Stroke* 13:601-611
- James IM (1968) Changes in cerebral blood flow and in systemic arterial pressure following spontaneous subarachnoid haemorrhage. *Clin Sci* 35:11-22
- Jones SC, Greenberg JH (1985) Evaluation of a double-tracer autoradiographic technique for the measurement of both local cerebral glucose metabolism and local cerebral blood flow. *J Cereb Blood Flow Metabol* 5:335-337
- Jones SC, Lear JL, Greenberg JH, Reivich MC (1979) A double label autoradiographic technique for the quantitative measure of cerebral blood flow and glucose metabolism. *Acta Neurol Scand (Suppl 72)* 60:202-203
- Kågstöm E, Granholm L, Rehncrona S (1973) Metabolite changes in CSF after subarachnoid hemorrhage. *Proceedings V International Congress of Neurological Surgery, Tokyo, Excerpta Medica* 293
- Kamiya K, Kuyama H, Symon L (1983) An experimental study of the acute stage of subarachnoid hemorrhage. *J Neurosurg* 59:917-924
- Logothetis J, Karacostas D, Karoutas G, Artemis N, Mansouri A, Milonas I (1983) A new model of subarachnoid hemorrhage in experimental animals with the purpose to examine cerebral vasospasm. *Exp Neurol* 81:257-278
- Martin WRW, Baker RP, Grubb RL, Raichle ME (1984) Cerebral blood volume, blood flow, and oxygen metabolism in cerebral ischemia and subarachnoid hemorrhage: an in-vivo study using positron emission tomography. *Acta Neurochir* 70:3-9
- Mickey B, Vorstrup S, Voldby B, Lindewald H, Harmsen A, Lassen NA (1984) Serial measurement of regional cerebral blood flow in patients with SAH using ¹³³Xe inhalation and emission computerized tomography. *J Neurosurg* 60:916-922
- Mies G, Niebuhr I, Hossmann KA (1981) Simultaneous measurement of blood flow and glucose metabolism by autoradiographic techniques. *Stroke* 12:581-588
- Miura M, Reis DJ (1969) Cerebellum: a pressor response elicited from fastigial nucleus and its efferent pathway in brain stem. *Brain Res* 13:595-599
- Mohr JP, Kase CS (1983) Cerebral vasospasm Part I. In cerebral vascular malformations. *Rev Neurol (Paris)* 139 (2):99-113
- Mraovitch S, Iadecola C, Reis DJ (1983) Vasoconstriction unassociated with metabolism in cerebral cortex elicited by electrical stimulation of the parabrachial nucleus in rat. *J Cereb Blood Flow Metabol* 3 (Suppl 1) 5:196-197
- Nakai M, Iadecola C, Ruggiero DA, Tucker LW, Reis DJ (1983) Electrical stimulation of cerebellar fastigial nucleus increases cerebral cortical blood flow without changes in focal metabolism: evidence for an intrinsic system in brain for primary vasodilation. *Brain Res* 260:35-49
- Owman Ch, Diemer NH (1985) Studies of LCBF and glucose metabolism in brain by computer assisted autoradiography. In *Quantitative Neuroanatomy in Transmitter Research* (eds K Fuxe, LF Agnati) London, MacMillan Press, pp. 71-87
- Pasqualin A, Vivenza C, Rizzotti P, Cocco C, Cavarazzani P, Da Pian R (1985) Serial biochemical changes in the cerebro-spinal fluid during the early stage of subarachnoid haemorrhage: relationship with cerebral vasospasm. In *Timing of*

- Aneurysm Surgery (ed LM Auer), Berlin, New York, Walter de Gruyter, pp 403-410
- Petruk KC, West GR, Marriott MR, McIntyre JW, Overton TA, Weir BKA (1972) Cerebral blood flow following induced subarachnoid hemorrhage in the monkey. *J Neurosurg* 37:316-324
- Powers WJ, Grubb RL, Baker RP, Mintun MA, Raichle MR (1985) Regional cerebral blood flow and metabolism in reversible ischemia due to vasospasm. *J Neurosurg* 62:539-546
- Reis DJ, Iadecola C, Nakai M (1985) Control of cerebral blood flow and metabolism by intrinsic neural systems in brain. In *Cerebrovascular Diseases* (eds. F. Plum, W. Pulsinelli), New York, Raven Press, pp 1-22
- Reivich M, Jehle J, Sokoloff L, Kety SS (1969) Measurement of regional cerebral flow with antipyrine- ^{14}C in awake cats. *J Appl Physiol* 27:296-300
- Sahlin Ch, Brismar J, Delgado T, Owman Ch, Salford LG, Svendgaard NAa (1986) Cerebrovascular and metabolic changes during the delayed vasospasm following experimental subarachnoid hemorrhage in baboons, and treatment with a calcium antagonist. Submitted for publication.
- Saito I, Shigeno T, Aritake K, Tanishima T, Sano K (1979) Vasospasm assessed by angiography and computerized tomography. *J Neurosurg* 51:466-475
- Sako K, Kato A, Diksic M, Yamamoto LY (1984) Use of short-lived ^{18}F and long-lived ^{14}C in double tracer autoradiography for simultaneous measurement of LCBF and LCGU. *Stroke* 15:896-900
- Sakurada O, Kennedy C, Jehle J, Brown JD, Carbin GL, Sokoloff L (1978) Measurement of local cerebral blood flow with iodo ^{14}C antipyrine. *Am J Physiol* 234(1):H59-H66
- Shannon DC, Kazemi H, Croteau N, Parsons EF (1970) Cerebral acid base changes during reduced cranial blood flow. *Resp Physiol* 8:385-396
- Shannon DC, Shore N, Kazemi H (1972) Acid-base balance in hemorrhagic cerebrospinal fluid. *Neurology* 22:585-589
- Simeone FA, Trepper PJ, Brown DJ (1972) Cerebral blood flow evaluation of prolonged experimental vasospasm. *J Neurosurg* 37:302-311
- Sokoloff L, Reivich M, Kennedy C, Des Rosiers MH, Patlak C, Pettigrew KD, Sakurada O, Shinohara M (1977) The ^{14}C -deoxyglucose method for the measurement of local cerebral glucose utilization: Theory procedure and normal values in the conscious and anesthetized albino rat. *J Neurochem* 28:897-917
- Sokoloff L (1978) Local cerebral energy metabolism: Its relationship to local functional activity and blood flow. In *Cerebral Vascular Smooth Muscle and Its Control*, Ciba Foundation Symposium 56 (eds. MJ Purves, K Elliott), New York, Elsevier/North Holland, pp. 171-197
- Solomon RA, Antunes JL, Chen RYZ, Bland L, Chien S (1985) Decrease in cerebral blood flow in rats after experimental subarachnoid hemorrhage: a new animal model. *Stroke* 16:58-64
- Sugi T, Fujishima M, Omae T (1975). Lactate and pyruvate concentrations, and acid-base balance of cerebrospinal fluid in experimentally induced intracerebral and subarachnoid hemorrhage in dogs. *Stroke* 6:715-719
- Tamura A, Graham DI, McCulloch J, Teasdale GMC (1981) Focal cerebral is-

- chemia in the rat:2. Regional cerebral blood flow determined by ^{14}C iodoantipyrine autoradiography following middle cerebral artery occlusion. *J Cereb Blood Flow Metabol* 1:61-69
- Umansky F, Kaspi T, Shalit MNC (1983) Regional cerebral blood flow in the acute stage of experimentally induced subarachnoid hemorrhage. *J Neurosurg* 58:210-216
- Wilkins RH (1977) The role of intracranial arterial spasm in the timing of operations for aneurysm. *Clinical Neurosurg* 24:185-207
- Voldby B, Enevoldsen EM, Jensen FT (1985) Regional CBF, intraventricular pressure, and cerebral metabolism in patients with ruptured intracranial aneurysms. *J Neurosurg* 62:48-58
- Yamori Y, Horie R, Handa H, Sato M, Fukase M (1976) Pathogenetic similarity of strokes in stroke-prone spontaneously hypertensive rats and humans. *Stroke* 7:46-53
- Zingesser LH, Schechter MM, Dexter J, Katzman R, Scheinberg LC (1968) On the significance of spasm associated with rupture of a cerebral aneurysm. *Arch Neurol* 18:520-528

Subarachnoid haemorrhage in the rat: effect on the development of vasospasm of selective lesions of the catecholamine systems in the lower brain stem

Niels Aage Svendgaard, M.D., Jan Brismar, M.D.*, Tia Juana Delgado, M.D., Evald Rosengren, M.D.**

Neurosurgical Research Department, Department of Neuroradiology and Department of Pharmacology**, University Hospital, Lund, Sweden.*

SUMMARY

Intracisternal injection of blood in the rat produces an angiographically demonstrable biphasic vasospasm. Lesioning at the level of the mesencephalon of the ascending catecholamine pathways from locus coeruleus in the pons and the A_1 and A_2 nuclei in the medulla oblongata prior to cisternal blood injection prevents the development of both acute and late spasm. Selective lesioning in the medulla oblongata of ascending fibres from A_1 and A_2 also prevents development of spasm, indicating that these nuclei, which project to the hypothalamus-pituitary, are essential for the spasm syndrome. It is suggested that a substance liberated either by the hypothalamus or by the pituitary is involved in the occurrence of spasm.

INTRODUCTION

Cerebral arterial vasospasm is one of the major complications of a subarachnoid haemorrhage (SAH) following the rupture of an intracranial aneurysm. Vasospasm can also occur after head injuries and intracranial operations^{1, 2}. The mechanism underlying the development of spasm is not known.

In a previous communication,³ we presented a vasospasm model in the rat. It was found that intracisternal injection of blood induced a reproducible biphasic spasm that could be evaluated by repeated angiography. The acute spasm was maximal at ten minutes and the late spasm maximal at two days. It has been reported earlier in both experimental and clinical studies that vasospasm has a biphasic time course⁴.

The role of the sympathetic system in vasospasm has been extensively investigated both in terms of etiology and in the therapy^{5, 6, 7, 8, 9, 10}. However, these studies have not identified the mechanism behind spasm or provided an effective treatment.

The role of the central catecholamine (CA) systems in spasm has not been investigated. In the present study we have examined the effect of lesioning of the central CA systems on the development of vasospasm following a SAH. It is shown that the A_2 and A_1 (nomenclature according to Dahlström and Fuxe)¹¹ nuclei in the medulla oblongata are essential for the development and maintenance of spasm.

MATERIAL AND METHODS

The experiments were performed on male Sprague-Dawley rats weighing between 300 and 420 g.

ANAESTHESIA AND SURGICAL PROCEDURES

The anaesthesia was initiated with 4% halothane. The animals were intubated and artificially ventilated. For the surgical procedure, anaesthesia was maintained with 0.75% halothane in a 70% nitrous oxide and 30% oxygen mixture. After infiltrating the skin with lidocain hydrochloride (xylocain®, Astra), a catheter was inserted into the axillary artery bilaterally for subsequent angiography. The femoral artery and vein were cannulated for continuous blood pressure monitoring and for infusion of drugs. (For technical details, see Delgado et al.)³. Heparin (Vitrum, 25 IU) was given i.v.. After surgery, the halothane was switched off and suxamethonium chloride (celocurin, Vitrum, 1 mg i.v.) was given. Thirty minutes were allowed to pass before the angiography.

ANGIOGRAPHY

Vertebro-basilar angiography was performed via bilateral axillary catheters. Metrizamide (Amipaque®, Nyegaard and Co., Oslo, Norway) was used as the contrast medium. The control angiography was followed by the injection of 0.3 ml of homologous blood intracisternally. The blood was injected via a previously implanted catheter connected to the cisterna magna³, and repeated angiography was performed. Measurements of the vertebral and basilar arteries were made using a technique similar to that described by Gabrielsen and Greitz¹². The diametres at four preselected points within the vertebro-basilar system were averaged and expressed as a percentage of the control values.

STEREOTAXIC LESIONS

A Kopf stereotaxic instrument for small animals was used. All lesions were made two to four weeks prior to the angiography. The animals were anaesthetized with Brietal (Lilly, 40 mg/kg i.p.).

LESION 1: BILATERAL LESIONS OF THE ASCENDING CA PATHWAYS IN THE MESENCEPHALON. The ascending CA fibres from the medulla oblongata and pons were lesioned chemically in the caudal mesencephalon rostral to the locus coeruleus and the subcoeruleus (Fig. 1 and 2, lesion 1). 6-hydroxydopamine (6-OHDA, 8 ug in 4 ul of saline containing 0.2 mg/ml of ascorbic acid) was injected bilaterally close to the dorsal tegmental bundle¹³. The following coordinates were used: 0.5 mm anterior to the interaural line, 1.1 mm lateral to the midline and 6.5 mm below the dura. The tooth-bar was kept at zero. The injection time was three minutes followed by another three minutes before withdrawal of the cannula. In sham lesioned animals, a cannula was lowered according to the same coordinates, but only the solvent was injected.

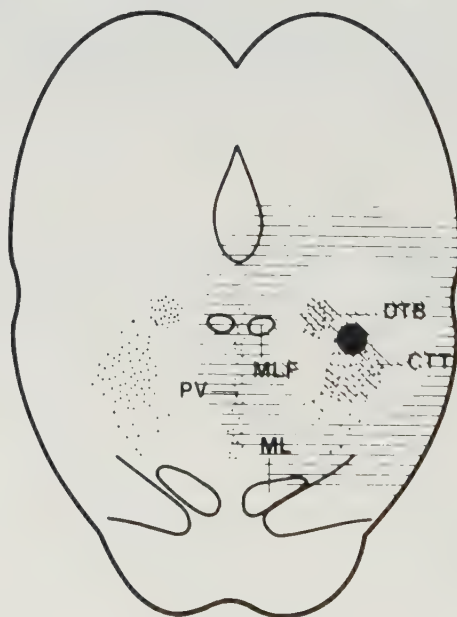


Fig. 1. Position of the bilateral intracerebral injection of 6-OHDA in the caudal mesencephalon. The figure illustrates, on one side, the area of unspecific damage (black circle), the area of the specific lesion of the ascending CA fibres (middle circle—skewed lines), and the area of estimated diffusion of the injected 6-OHDA (outer circle—horizontal lines)^{13,16}. CTT central tegmental tract; DTB dorsal tegmental bundle; ML medial lemniscus; MLF longitudinal medial fasciculus; PV paramedian NA fibre flow.

LESION 2: BILATERAL LESIONS OF THE ASCENDING CA PATHWAYS IN THE MEDULLA OBLONGATA. The ascending CA fibres from A₁ and A₂ were lesioned chemically in the medulla oblongata (Fig. 2, lesion 2). 6-OHDA (3 ug in 1.5 ul of saline containing 0.2 mg/ml of ascorbic acid) was injected at two levels; 7.2 and 8 mm below the dura. The other coordinates were: 2.3 mm caudal to the interaural line and 1.1 mm lateral to the midline. The toothbar was kept at zero. In the sham lesioned animals, the cannula was lowered to the same coordinates, but only the solvent was injected.

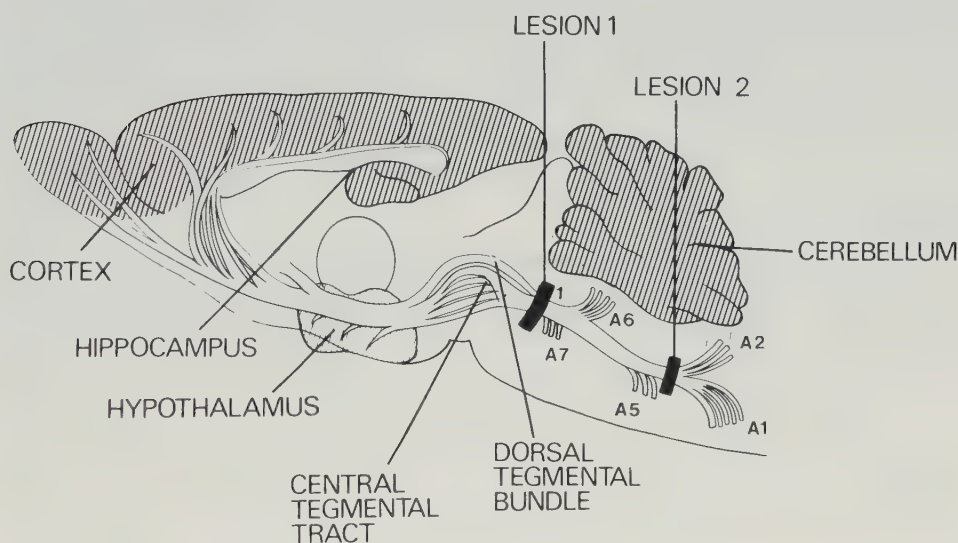


Fig. 2. Schematic illustration of the CA pathways in the rat brain showing the two different lesion sites.

CONTROL OF THE LESIONS

The lesion of the ascending CA pathways in the mesencephalon was evaluated chemically and with fluorescence histochemistry. The frontal cortex and the diencephalon were examined for the content of noradrenaline (NA) using liquid chromatography with electrochemical detection. The NA content of the frontal cortex was reduced

by $97 \pm 1.7\%$ (Mean $\pm$ SEM). The NA content in the diencephalon was reduced by $87.3 \pm 6.4\%$. For the fluorescence histochemistry the animals were perfused according to the method of Lorén et al.¹⁴ The frontal cortex and the diencephalon were examined. There was a complete denervation of the cortex and a marked denervation of the hypothalamus.

The lesion of the ascending CA pathways in the medulla oblongata was controlled by chemical analysis of the NA content in the frontal cortex and the diencephalon, and with fluorescence microscopy of the lesion site. There was no significant reduction in the NA content of the frontal cortex. In the diencephalon the NA content was reduced by $65.9 \pm 5.1\%$. The microscopy confirmed that the lesions were correctly placed and that the locus coeruleus was intact.

EXPERIMENTAL DESIGN.

All angiographical examinations were carried out between two and four weeks after the stereotaxic lesions. The animals had control angiography followed by the intracisternal injection of blood. Repeat angiography was made ten minutes and two days post SAH.

GROUP I. LESION OF THE ASCENDING CA PATHWAYS IN THE MESENCEPHALON.

Six animals had angiography pre and post SAH.

Three of the six animals were perfused for fluorescence microscopy. In the other three, the NA content in the frontal cortex and the diencephalon was determined.

GROUP II. LESIONS OF THE ASCENDING CA PATHWAYS IN THE MEDULLA OBLONGATA.

Six animals had angiography pre and post SAH.

In all six animals, the NA content in the frontal cortex and the diencephalon was measured. Fluorescence microscopy of the lesion site and the locus coeruleus area was made in three of the animals.

GROUP III. SHAM LESIONS.

There were three animals with sham lesions in the mesencephalon and three animals with sham lesions in the medulla oblongata. All

animals were examined with angiography, both pre and post SAH. Chemical determination of the NA content in the frontal cortex and the diencephalon was made in all the animals.

GROUP IV. NORMAL ANIMALS.

Six animals had angiography pre and post SAH. Chemical determination of the NA content in the frontal cortex and the diencephalon was made in all the animals.

STATISTICAL ANALYSIS

The data were evaluated statistically with the Student t-test.

RESULTS

The physiological parameters in the experimental groups are shown in Table I. There was no inter-group difference in the sham lesioned animals and the data from these animals were therefore pooled. The control mean arterial blood pressure (MABP) in animals with medullary lesions was significantly lower as compared to sham lesioned animals ($p < 0.05$). Immediately after the intracisternal injection, there was an increase in MABP in all the groups. The MABP was still elevated in the animals with medullary lesions ten minutes after the blood injection. It was significantly higher than the control value ($p < 0.01$). The pH values were close to 7.4. The PaO_2 values were about 150 mmHg, and the PaCO_2 values were around 37. The temperature was kept close to 37°C .

TABLE I

Physiological parameters pre and post SAH: Influence of lesions of the central CA systems. Values are means $\pm$ S.E.M.

ANIMALS		MAP mmHg	PULSE	pH	PaO ₂	PaCO ₂	N
Normal	control	121 $\pm$ 4	305 $\pm$ 29	7.41 $\pm$ 0.01	156 $\pm$ 10	35.7 $\pm$ 1.3	6
	10 min	118 $\pm$ 8	282 $\pm$ 18	7.41 $\pm$ 0.01	156 $\pm$ 10	35.7 $\pm$ 1.3	6
	2 days	111 $\pm$ 8	300 $\pm$ 35	7.41 $\pm$ 0.02	156 $\pm$ 15	36.5 $\pm$ 1.2	6
sham	control	126 $\pm$ 4	314 $\pm$ 11	7.37 $\pm$ 0.02	151 $\pm$ 7	37.2 $\pm$ 1.1	6
	10 min	121 $\pm$ 8	317 $\pm$ 17	7.34 $\pm$ 0.02	150 $\pm$ 10	37.7 $\pm$ 1.0	6
	2 days	119 $\pm$ 7	290 $\pm$ 24	7.44 $\pm$ 0.03	155 $\pm$ 12	36.8 $\pm$ 1.3	6
bilateral lesions in the mesen- cephalon	control	121 $\pm$ 4	321 $\pm$ 13	7.39 $\pm$ 0.03	149 $\pm$ 9	37.7 $\pm$ 1.7	6
	10 min	120 $\pm$ 5	310 $\pm$ 10	7.39 $\pm$ 0.03	149 $\pm$ 9	37.7 $\pm$ 1.7	6
	2 days	114 $\pm$ 9	293 $\pm$ 26	7.43 $\pm$ 0.03	159 $\pm$ 15	37.3 $\pm$ 1.8	6
bilateral lesions in the medulla oblongata	control	108 $\pm$ 6 *	295 $\pm$ 26	7.38 $\pm$ 0.01	154 $\pm$ 8	38.3 $\pm$ 1.2	6
	10 min	131 $\pm$ 5 **	255 $\pm$ 15	7.39 $\pm$ 0.02	146 $\pm$ 8	38.2 $\pm$ 1.3	6
	2 days	114 $\pm$ 8	252 $\pm$ 12	7.42 $\pm$ 0.03	140 $\pm$ 5	38.0 $\pm$ 1.8	6

*p<0.05, **p<0.01, ***p<0.001

In both normal and sham lesioned animals, cisternal blood injection produced an acute spasm of about 40% at ten minutes and a late spasm of about 25% at two days post SAH (Fig.3).

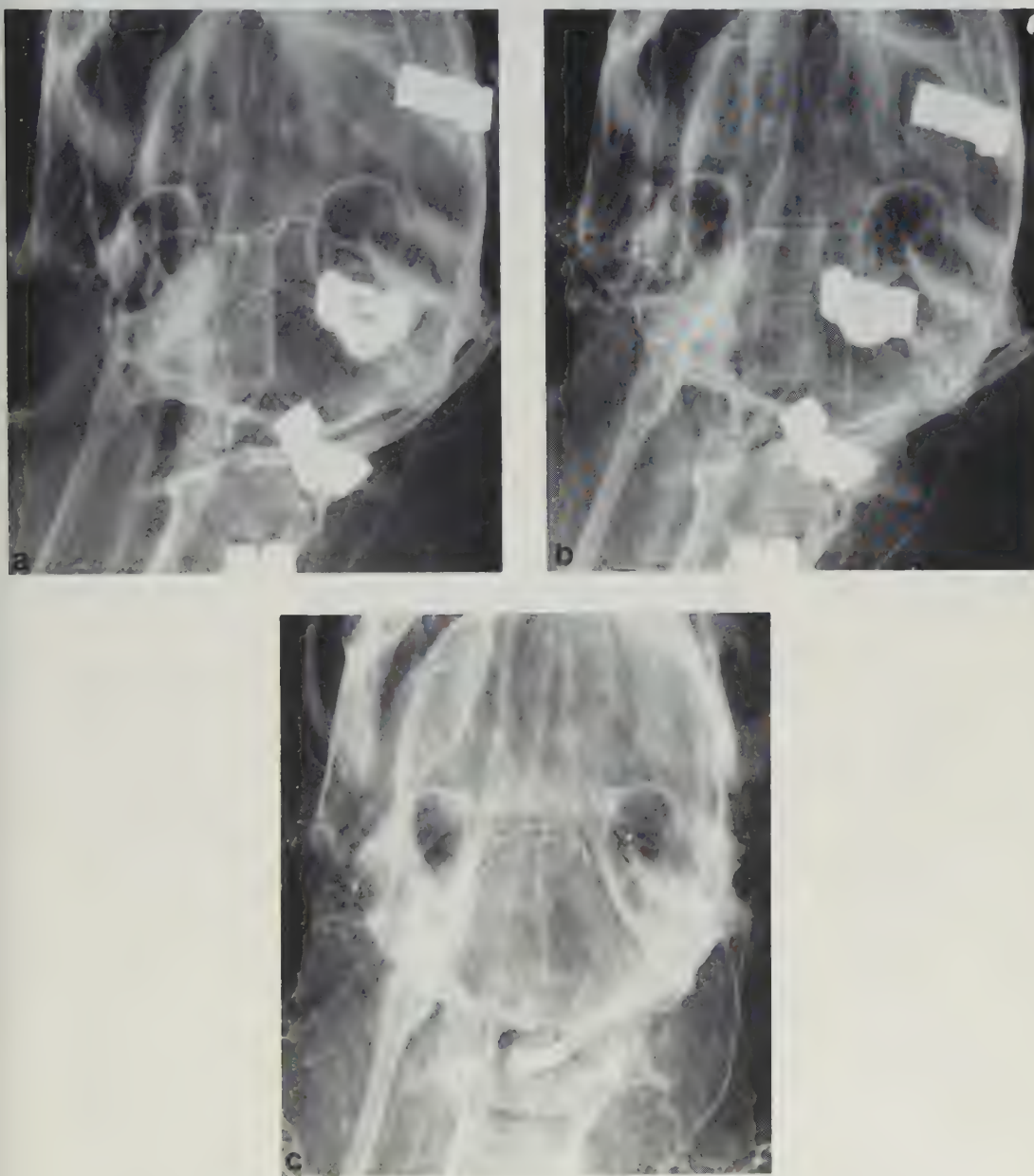


Fig. 3. Vertebral-basilar angiography in a sham lesioned animal. A, control. B and C, ten minutes and two days respectively, after cisternal blood injection. Spasm is seen at both ten minutes and two days post SAH.

Lesioning of the ascending CA pathways in the medulla oblongata or in the mesencephalon in advance of the SAH completely prevented the development of both the acute and the late spasm (Fig. 4A and B and Fig. 5). The pre SAH mean vessel diameter of the vertebro-basilar system in the lesioned animals did not differ from that seen in sham lesioned or normal animals.

No paralysis was noted in any of the animals after the SAH. The normal and the sham lesioned animals were noticeably drowsy after blood injection. In contrast, the animals with lesions in the mesencephalon or medulla oblongata seemed unaffected by the SAH.

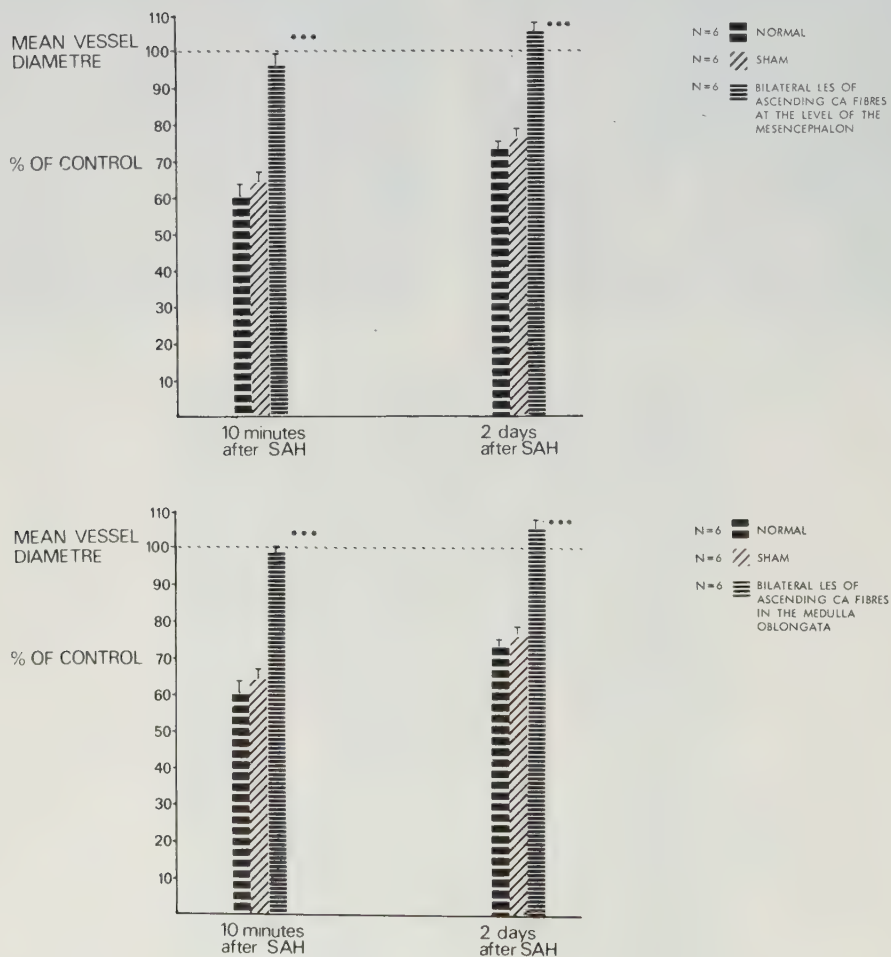


Fig. 4 A and B. Angiographical changes in vertebro basilar diameter post SAH after lesions in the mesencephalon (A) and medulla oblongata (B). The values are means $\pm$ SEM in per cent of control.

*p<0.05, **p<0.01, ***p<0.001.

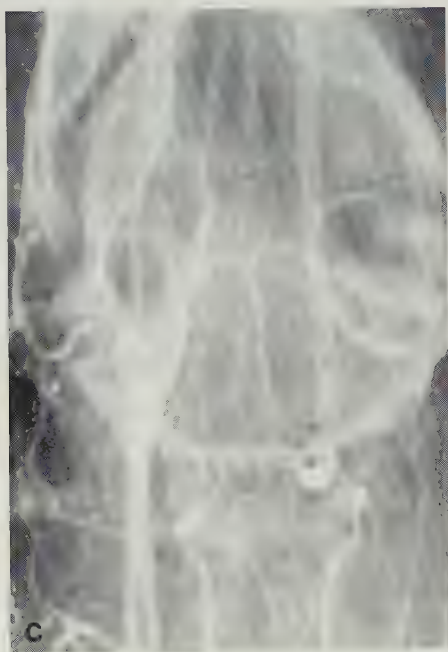


Fig. 5. Vertebro-basilar angiography in an animal with bilateral lesions of the ascending CA pathways in the medulla oblongata. A, control. B and C, ten minutes and two days respectively, after cisternal blood injection. No spasm is seen at ten minutes and two days post SAH.

DISCUSSION

The present study demonstrates that chemical lesioning of the ascending CA pathways in the mesencephalon or in the medulla oblongata in the rat prior to cisternal blood injection completely prevents the development of both acute and late spasm. Repeated angiography after a cisternal blood injection in normal animals has shown an acute spasm maximal at ten minutes and a late spasm maximal at two days post SAH³.

LESIONS.

The lesions were produced by 6-OHDA. This compound has a selective destructive effect on CA axons and terminals, leaving neurons containing other transmitters unaffected^{15,16}. Lesioning of the ascending CA fibres in the mesencephalon has been found to cause a reduction in the NA content of the frontal cortex of between 89 and 100 %^{17,18}; in this study the reduction was 97%. Lesioning of the ascending CA pathways in the medulla oblongata has to our knowledge not been described earlier. In our study there was no significant reduction in the NA content of the frontal cortex with this lesion. In this group, fluorescence microscopy showed that locus coeruleus, which innervates the frontal cortex, was intact.

ANATOMY.

The CA containing nuclei in the pons and medulla oblongata giving rise to ascending projections in the brain stem include locus coeruleus or A₆ and A₁ and A₂^{11,19}. The locus coeruleus is located dorsomedially in the floor of the rostral part of the fourth ventricle in the pons. These neurons, probably all NA containing, project via the dorsal tegmental bundle to the hypothalamus, cortex and hippocampus²⁰.

A₁ is an elongated accumulation of neurons located ventrolaterally in the medulla oblongata at the level of the lateral reticular nucleus. A₂ is a V-shaped accumulation of cells situated dorsomedially in the medulla oblongata. The base of the V is located caudal to the obex in the commissural nucleus, and the sides project rostrally within the medial part of the nucleus tractus solitarius. The A₁ and

A₂ nuclei are predominantly NA containing. However, immunofluorescence studies indicate that adrenaline producing cells are present in the rostral part of these cell groups²¹. Dopamine producing cells have also been described in A₂^{22,23}. The A₁ and A₂ nuclei project to the diencephalon, providing the main CA innervation to the hypothalamus-pituitary^{24,25}. Finally, the three nuclei are interconnected and they probably all send fibres to the spinal cord¹⁹.

PHYSIOLOGY.

The functional significance of the CA containing nuclei in the lower brain stem is only poorly understood. Locus coeruleus has been suggested to participate in the cardiovascular regulation either via its descending projections to the nucleus tractus solitarius²⁶ and the spinal cord²⁷ or via ascending projections to the hypothalamus^{24,28}. Also, it has been shown to have an inhibitory effect on the neurons in the cortex²⁹, cerebellum³⁰ and hippocampus³¹. In addition, fibres originating in the locus coeruleus have been seen in close association with the microvessels in the brain parenchyma^{32,33,34}. This has led to investigations into the role of the locus coeruleus in cerebral blood flow and metabolism. To date, studies have not revealed a major influence of the locus coeruleus on cerebral blood flow and metabolism under normal conditions^{35,36}.

The connections of the A₁ and A₂ nuclei suggest an involvement in autonomic regulation, especially in the regulation of systemic blood pressure^{37,38,39}. The adrenaline containing neurons in the rostral part of A₁ have been shown to exert a tonic vasomotor control on the peripheral vasculature through their connections to the thoracic intermediolateral cell column³⁸. On the other hand, the NA containing neurons in the caudal part of A₁ has been found to inhibit this vasomotor tone⁴⁰. The NA containing neurons in A₂ have been found to modulate the baroreceptor reflex^{39,41}. Animals with lesions of A₂ demonstrate an increased lability and reactivity in blood pressure to stimuli³⁹. Stimulation within the dorsal reticular formation has been found to induce alterations in cerebral blood flow^{42,43}.

Finally, NA neurons in A₁, A₂ and A₆ have been suggested to influence the release of hormones from the anterior and posterior pituitary^{24,44}.

POSSIBLE MECHANISMS BEHIND VASOSPASM.

The changes in MABP seen both before and ten minutes after the SAH in animals with medullary lesions are not responsible for the absence of vasospasm. Blood pressure changes of this magnitude do not alter the degree of angiographical spasm (unpublished observations). An explanation for the lower blood pressure pre SAH in these animals is that 6-OHDA affects the rostral part of the A_1 nuclei which is known to be involved in the maintenance of peripheral vascular tone³⁸. The increase in MABP seen ten minutes after the SAH might reflect an exaggerated response of the blood pressure to a stimulus. This exaggerated response could be due to an impairment of the connection between the rostral A_1 and A_2 .

It has been widely believed that blood or its degradation products directly produce vasospasm, in particular the acute phase after a SAH^{45,46}. The absence of spasm in the lesioned animals after a cisternal blood injection, speaks against this assumption. The present data suggest that a neural or neurohormonal mechanism is involved. Cerebral vasospasm was prevented by both the mesencephalic and the medullary lesions. The lesions in the mesencephalon destroy the ascending fibres from locus coeruleus running in the dorsal tegmental tract, and the ascending fibres from A_1 and A_2 projecting via the central tegmental tract. The lesions in the medulla oblongata only destroy the ascending fibres from A_1 and A_2 . Accordingly, the ascending projections from A_1 and/or A_2 are those involved in the development of spasm.

How are the A_1 and/or A_2 nuclei involved in the development of spasm? To discuss this question one could consider the afferents and efferents of these nuclei. However, nothing is known about the afferents to A_1 . On the other hand afferents from the trigeminal and facial nerves are known to terminate in an area of the nucleus tractus solitarius containing A_2 neurons. It is interesting that a trigeminal innervation of the circle of Willis and the anterior and middle cerebral arteries has been described⁴⁷. Similar observations have been made in our laboratory using antero- and retrograde tracing techniques (to be published). Subarachnoid blood might stimulate sensory nerves on the cerebral vessels or in cerebral tissue setting in motion via A_2 the events which lead to development of spasm.

There are efferents from A_1 and A_2 projecting to the hypothalamus-pituitary. The hypothalamus could be involved in the devel-

opment of spasm in three different ways: Via (1) the autonomic nervous system or (2) the anterior or posterior pituitary or (3) the release of hormones into the cerebrospinal fluid from hypothalamic neurons⁴⁸. A role for the hypothalamus in the spasm syndrome has been suggested earlier⁴⁹.

An autonomic mechanism mediated via the adrenal medulla or via the superior cervical ganglion is unlikely. In separate experiments we have injected blood intracisternally in animals with bilateral adrenal demedullation or cervical ganglionectomy and found that both acute and late spasm still developed⁵⁰.

In conclusion: The A₂ and/or A₁ cell groups are essential for the development and maintenance of vasospasm after a SAH via their projections to the hypothalamus-pituitary.

ACKNOWLEDGEMENT

Supported by grants from the Anna-Lisa and Sven-Eric Lundgren Foundation; Elsa Schmitz Foundation; Thorsten and Elsa Segerfalk Foundation and the Swedish Medical Research Council (NO 04X-5300 and 14X-732).

REFERENCES

1. Wilkins RH: Intracranial vascular spasm in head injuries. In Vinken PJ, Bruyn GW, Brookman R (eds), *Handbook of Clinical Neurology*, Vol 23. *Injuries of the Brain and Skull*, Part 1. Amsterdam: North Holland, pp 163-197, 1975.
2. Mohr JP, Kase CS: Cerebral vasospasm Part 1. In cerebral vascular malformations. *Rev Neurol (Paris)* 139(2):99-113, 1983
3. Delgado TJ, Brismar J, Svendgaard N Aa: Subarachnoid haemorrhage in the rat: Angiography and fluorescence microscopy of the major cerebral arteries. *Stroke* 16:595-602, 1985
4. Heros RC, Zervas NT, Negoro M: Cerebral vasospasm. *Surg Neurol* 5:354-362, 1976.
5. Fraser RA, Stein BM, Barrett RE: Noradrenergic mediation of experimental cerebrovascular spasm. *Stroke* 1:356-362, 1970.
6. Kodama N, Hari S, Kamiyama K, Mizoi K, Suzuki J: Cervical sympathectomy for cerebral vasospasm after aneurysm rupture. In: Wilkins RH (ed), *Cerebral Arterial Spasm: Proceedings of the Second International Workshop*, Amsterdam. Baltimore/London: Williams and Wilkins, pp 680-684, 1980.

7. Nagai H, Noda S, Mabe H: Experimental cerebral vasospasm Part 2: Effects of vasoactive drugs and sympathectomy on early and late spasm. *J Neurosurg* 42:420-428, 1975.
8. Peerless SJ, Kendall MJ: Experimental cerebral vasospasm. In Whisnant JP, Sandok BA (eds). *Proceedings of the Ninth Princeton Conference on Cerebral Vascular Disease*. New York: Grune & Stratton, pp 49-58, 1974.
9. Sundt TM, Onofrio BM, Meredith J: Treatment of cerebral vasospasm from SAH with isoproterenol and lidocaine hydrochloride. *J Neurosurg* 38:557-560, 1973.
10. Svendgaard N Aa, Edvinsson L, Olin T, Owman Ch, Sahlin Ch: On the pathophysiology of cerebral vasospasm: Transmitter changes in perivascular sympathetic nerves, and increased pial artery sensitivity to norepinephrine and serotonin. In Owman Ch, Edvinsson L (eds), *Neurogenic Control of the Brain Circulation*, Oxford: Pergamon Press, pp 143-152, 1977.
11. Dahlström A, Fuxe K: Evidence for the existence of monoamine containing neurons in the central system. I. Demonstration of monoamines in the cell bodies of brain stem neurones. *Acta physiol Scand* 62 (Suppl. 232):1-55, 1964.
12. Gabrielsen TO, Greitz T: Normal size of the internal carotid, middle cerebral and anterior cerebral arteries. *Acta Radiol (Diagn)* 10:1-10, 1970.
13. Agid Y, Javoy F, Glowinski J, Bouvet D, Sotelo C: Injection of 6-hydroxydopamine into the substantia nigra of the rat. II. Diffusion and specificity. *Brain Res* 58:291-301, 1973.
14. Loren I, Björklund A, Falck B, Lindvall O: An improved formaldehyde histofluorescence procedure for freeze-dried paraffin-embedded tissue based on combined formaldehyde-glyoxylic acid perfusion with high magnesium content and acid pH. *Histochem* 49:177-192, 1976.
15. Tranzer JP, Thoenen H: Ultramorphologische Veränderungen der Sympathischen Nervenendigungen der Katze nach Vorbehandlung mit 5und 6-hydroxydopamine. *Naynun Schmiedebergs Arch Pharmac Exp Path* 257:343-344, 1967.
16. Ungerstedt U: Effect of 6-hydroxydopamine on the monoamine- containing neurons in the CNS. Histochemical studies on the effect of intracerebral and intraventricular injections of 6-hydroxydopamine on monoamine neurons in the rat brain. In Malmfors T, Thoenen H (eds) *6-Hydroxydopamine and Catecholamine Neurons*. Amsterdam: North Holland, pp 101-127, 1971.
17. Norberg K, Scatton B, Korf J: Amphetamine-induced increase in rat cerebral blood flow: apparent lack of catecholamine involvement. *Brain Res* 149:165-174, 1978.
18. Westerberg V, Lewis J, Corcoran ME: Depletion of noradrenaline fails to affect kindled seizures. *Exp Neurol* 84:237-240, 1984.
19. Lindvall O, Björklund A: Dopamine- and norepinephrine-containing neuron systems: Their anatomy in the rat brain. In Emson ICP (ed), *Chemical Neuroanatomy*, New York: Raven Press, pp 229-255, 1983.
20. Amaral DG, Sinnamon HM: The locus coeruleus: Neurobiology of a central noradrenergic nucleus. *Progress in Neurobiol.* 9:147-196, 1977.
21. Hökfelt T, Fuxe K, Goldstein M, Johansson O: Immunohistochemical evidence for the existence of adrenaline neurons in the rat brain. *Brain Res* 66:235-251, 1974.

22. Armstrong DM, Ross CA, Pickel VM, Joh TH, Reis DJ: Distribution of dopamine-, noradrenaline-, and adrenaline-containing cell bodies in the rat medulla oblongata: demonstrated by the immunocytochemical localization of catecholamine biosynthetic enzymes. *J Comp Neurol* 212:173-187, 1982.
23. Versteeg DHG, Van der Gugten J, DeJong W, Palkovits M: Regional concentrations of noradrenaline and dopamine in rat brain. *Brain Res* 113:563-574, 1976.
24. Sawchenko PE, Swanson LW: The organization of noradrenergic pathways from the brainstem to the paraventricular and supraoptic nuclei in the rat. *Brain Res Rev* 4:275-325, 1982.
25. Palkovits M, Zaborszky L, Feminger A, Herman JP, Kanyicska B, Szabo D: Noradrenergic innervation of the rat hypothalamus: experimental, biochemical and electron microscopic studies. *Brain Res* 191:161-171, 1980.
26. Takahashi Y, Satoh K, Sakumoto T, Tohyama M, Shimizu N: A major source of catecholamine terminals in the nucleus tractus solitarii. *Brain Res* 172:372-377, 1979.
27. Nygren LG, Olson L: A new major projection from locus coeruleus: the main source of noradrenergic nerve terminals in the ventral and dorsal columns of the spinal cord. *Brain Res* 132:85-93, 1977.
28. Przuntek H, Philippu A: Reduced pressor responses to stimulation of the locus coeruleus after lesion of the posterior hypothalamus. *Naunyn-Schmiedeberg's Arch Pharmacol* 276:119-122, 1973.
29. Phillis JW, Kostopoulos GK: Activation of a noradrenergic pathway from the brain stem to rat cerebral cortex. *Gen Pharmacol* 8: 207-211, 1977.
30. Hoffer BJ, Siggins GR, Oliver AP, Bloom FE: Activation of the pathway from locus coeruleus to rat cerebellar Purkinje neurons: pharmacological evidence of noradrenergic central inhibition. *J Pharmacol Exp Ther* 181:553-569, 1973.
31. Segal M, Bloom FE: The action of norepinephrine in the rat hippocampus. III. The effects of locus coeruleus stimulation in the awake rat. *Brain Res* 107:499-511, 1976.
32. Edvinsson L, Lindvall M, Nielsen KC, Owman CH: Are brain vessels innervated also by central (non-sympathetic) adrenergic neurones? *Brain Res* 63: 496-499, 1973.
33. Hartman BK, Zide D, Udenfriend S: The use of dopamine beta- hydroxylase as a marker for the central noradrenergic nervous system in rat brain. *Proc Nat Acad Sci (Wash)* 69:2722-2726, 1972.
34. Rennels ML, Forbes MS, Anders JJ, Nelson E: Innervation of the microcirculation in the central nervous system and other tissues. In Owman Ch, Edvinsson L (eds), *Neurogenic Control of the Brain Circulation*, Oxford: Pergamon Press, pp 91-104, 1977.
35. Dahlgren N, Lindvall O, Sakabe T, Stenevi U, Siesjö BK: Cerebral blood flow and oxygen consumption in the rat brain after lesions of the noradrenergic locus coeruleus. *Brain Res* 209:11-23, 1981.
36. Savaki HE, Graham DI, Grame JJ, McCulloch J: Functional consequences of unilateral lesion of the locus coeruleus: A quantitative ¹⁴C 2-deoxyglucose investigation. *Brain Res* 292:239-249, 1984.

37. Alexander RS: Tonic and reflex functions of medullary sympathetic cardiovascular centers. *J Neurophysiol* 9:205-217, 1946.
38. Ross CA, Ruggiero DA, Joh TH, Park DH, Reis DJ: Adrenaline synthesizing neurons in the rostral ventrolateral medulla: a possible role in tonic vasomotor control. *Brain Res* 273:356-361, 1983.
39. Talman WT, Snyder D, Reis DJ: Chronic lability of arterial pressure produced by destruction of A_2 catecholaminergic neurons in rat brainstem. *Circ Res* 46:842-853, 1980.
40. Blessing WW, Reis DJ: Inhibitory cardiovascular functions of neurons in the caudal ventrolateral medulla of the rat: relationship to the area containing A_1 noradrenergic cells. *Brain Res* 253:161-171, 1982.
41. DeJong W, Nijkamp FP: Hypotensive action of noradrenaline and α -methyl-noradrenaline in the area of the nucleus tractus solitarius in the rat brainstem. In Davies DS, Reid JL (eds) *Central Action of Drugs in Blood Pressure Regulation*, London: Pitman, pp 179-180, 1975.
42. Reis DJ: Nervous control of the cerebral blood flow in normal health and in relationship to cerebrovascular disease. In Castaigne P, Lhermitte F, Gauthier JC, Morinier A (eds) *Maladies Vasculaires Cerebrales (Cerebrovascular Diseases)*, Paris: Baillier, pp 197-224, 1979.
43. Iadecola C, Nakai M, Arbit E, Reis DJ: Global cerebral vasodilatation elicited by focal electrical stimulation within the dorsal medullary reticular formation in anesthetized rat. *J Cereb Blood Flow Metabol* 3: 270-279, 1983.
44. Weiner RI, Ganong WG: Role of brain monoamines and histamine in regulation of anterior pituitary secretion. *Physiol Rev* 58:905-976, 1978.
45. Echlin FA: Spasm of basilar and vertebral arteries caused by experimental subarachnoid hemorrhage. *J Neurosurg* 23:1-11, 1965.
46. Zervas NT, Kuwayama A, Rosoff CB, Salzman EW: Cerebral arterial spasm-modification by inhibition of platelet function. *Arch Neurol* 28:400-404, 1973.
47. Mayberg MR, Zervas NT, Moskowitz MA: Trigeminal projections to supratentorial pial and dural blood vessels in cats demonstrated by horseradish peroxidase histochemistry. *J Comp Neurol* 223:46-56, 1984.
48. Pollay M: Recent developments in cerebrospinal fluid physiology. *Contemp Neurosurg* 1 (14):1-6, 1979.
49. Wilkins RH: Hypothalamic dysfunction and intracranial arterial spasm. *Surg Neurol* 4:472-480, 1975.
50. Svendgaard N Aa, Brismar J, Delgado TJ, Diemer NH: The influence of the peripheral and central monoamine systems on the development of cerebral vasospasm in the rat. *Neurol Res* 7:30-34, 1985.

Subarachnoid haemorrhage in the rat: Cerebral blood flow and glucose metabolism after selective lesions of the catecholamine systems in the brain stem.

Tia J Delgado, *Nils H Diemer , Niels-Aage Svendgaard

*From the Neurosurgical Research Department, University Hospital, Lund, Sweden and the *Neuropathological Institute, University of Copenhagen, Denmark*

SUMMARY

A double isotope autoradiographic technique was used to evaluate cerebral blood flow and glucose metabolism two days after a subarachnoid haemorrhage (SAH) in rats with lesions in the lower brain stem. Lesioning in the mesencephalon of the ascending catecholamine (CA) pathways from locus coeruleus and from the A_1 and A_2 nuclei, or lesioning in the medulla oblongata of the ascending fibres from A_1 and A_2 prevents the development of the global changes in flow and metabolism seen in normal animals post SAH. Also, the focal low flow areas with markedly elevated deoxyglucose uptake, which can develop in normal animals two days post SAH, were not seen in the lesioned animals after the SAH. The findings indicate that the A_1 and A_2 nuclei, which project to the hypothalamus-pituitary, are essential for the flow and metabolic changes after a SAH. The lesions per se did not change baseline flow and metabolism as compared to sham lesioned animals.

INTRODUCTION

The concept that the cerebral circulation could be influenced by a central mechanism was proposed by Luys (1879). An experimental study by LeBeau and Rabinovitch (1949) suggested that the hypothalamus is involved in the regulation of the cerebral circulation. Subsequently, Ingvar and Söderberg (1958) demonstrated by stimulation experiments that areas within the lower brain stem can alter cerebral blood flow (CBF). Recently it has been shown that stimulation of discrete areas within the parabrachial nucleus in the pons can alter CBF with or without parallel changes in metabolism (Mraovitch et al. 1983; Reis et al. 1985). Further, Iadecola et al. (1983) have shown that a center in the dorsal medullary reticular formation can change cerebral metabolism with secondary effects on flow.

Pool (1958) in his discussion of possible mechanisms behind cerebral vasospasm suggested that the brain stem could play a role in the development of spasm. In a previous communication (Svendgaard et al. 1985), using a rat SAH model, we have shown that nuclei in the lower brain stem play an essential role in the development of spasm post SAH. The aim of the present study is to investigate if brain stem centers are also involved in the CBF and metabolic changes

which occur after a SAH (Grubb et al. 1977; Delgado et al. 1986; Sahlin et al. 1986).

MATERIAL AND METHODS

The experiments were performed on male Sprague-Dawley rats (SPF strain, Møllegaards Avlslaboratorium, Køge, Denmark) weighing between 260 and 320 g. The animals were fasted for 12-18 hours before the CBF and/or CMRglu studies.

STEREOTAXIC LESIONS

A Kopf stereotaxic frame was used. The lesions were made under barbiturate anaesthesia (Brietal®, Lilly; 40 mg/kg i.p.).

BILATERAL LESIONS OF THE ASCENDING CA PATHWAYS IN THE MESENCEPHALON. The ascending CA fibres from the medulla oblongata and pons were lesioned at the level of the caudal mesencephalon rostral to the locus coeruleus and subcoeruleus area (Fig 1, lesion 1). 6-Hydroxydopamine (6-OHDA; 8 ug in 4 ul of saline containing 0,2 mg/ml ascorbic acid) was injected bilaterally. With the toothbar at zero, the following coordinates were used for the injections: 0,5 mm rostral to the interaural line, 1,1 mm lateral to the midline and 6,5 mm down from the dura. In the sham lesioned animals, only the solvent was injected.

BILATERAL LESIONS OF THE ASCENDING CA FIBRES FROM A₁ AND A₂ IN THE MEDULLA OBLONGATA (nomenclature according to Dahlström and Fuxe 1964). These ascending CA fibres were lesioned in the medulla oblongata (Fig 1, lesion 2). The lesions were produced by injecting 6-OHDA (3 ug in 1,5 ul of saline containing 0,2 mg/ml ascorbic acid) at 7,2 and 8 mm below the dura. The toothbar was kept at zero and the coordinates were as follows: 2,3 mm caudal to the interaural line and 1,1 mm lateral to the midline. In the sham lesioned animals, only the solvent was injected.

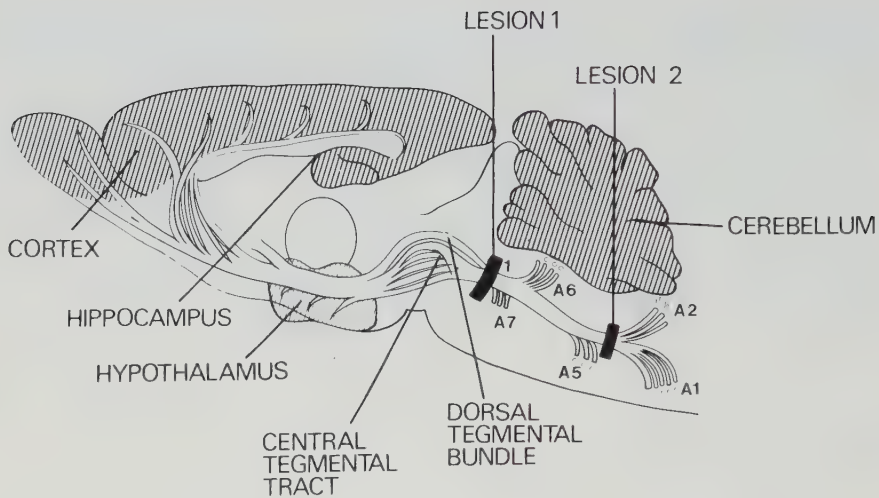


Fig 1: Schematic illustration of the CA pathways in the rat brain showing the mesencephalic (lesion 1) and the medullary (lesion 2) lesion sites.

CONTROL OF LESIONS.

The mesencephalic lesions were controlled by measuring the noradrenaline (NA) content in the frontal cortex. NA was determined using liquid chromatography with electrochemical detection (Eriksson and Persson 1982). Only animals with a reduction in NA of more than 95 per cent were included in the study.

The medullary lesions were also controlled by measuring the NA content of the frontal cortex. Only animals with no significant reduction in NA were included in the study.

In the animals with sham mesencephalic or medullary lesions the NA content in the frontal cortex was similar to that seen in normal animals.

EXPERIMENTAL SAH MODEL.

Under ether anaesthesia, homologous blood (0,07 ml) was injected into the cisterna magna via a previously implanted catheter (Delgado et al. 1985). The catheter was inserted three to seven days before the blood injection.

SIMULTANEOUS CBF AND CMRglu MEASUREMENTS.

Determinations of local CBF (Reivich et al. 1969; Sakurada et al. 1978, Gjedde et al. 1980) and CMRglu (Sokoloff et al. 1977) were made in the same animal using ^{14}C -iodoantipyrine and ^3H -deoxyglucose respectively, according to the double autoradiographic method of Diemer and Rosenørn (1981). For technical details see Delgado et al. (1986). The animals were anaesthetized with 4% halothane, intubated and then artificially ventilated with a 70% nitrous oxide and a 30% oxygen mixture. During cannulation of both femoral arteries and veins, 0,75% halothane was added to the gas mixture. After completion of the surgery, the halothane was switched off and at least 30 minutes were allowed to pass before the measurements. The mean arterial blood pressure (MABP) was continuously monitored and blood gases were regularly controlled. The temperature was kept close to 37°C .

^3H -deoxyglucose (750 uCi) was infused intravenously in 30 seconds. Blood samples were taken repeatedly over 45 minutes for determination of the plasma deoxyglucose integral. Immediately after the last sample, 60 uCi of ^{14}C -iodoantipyrine dissolved in 0,25ml 0,9% saline was given intravenously as a bolus injection. Over 20 seconds arterial blood was withdrawn using a constant velocity withdrawal pump for the determination of the ^{14}C -iodoantipyrine integral. At the end of the 20 seconds, the animal was decapitated and the brain frozen for later sectioning.

Brain radioactivity content was measured using autoradiography of 20 μm sections. Sections for detection of ^3H -deoxyglucose content were washed immediately after cutting, 2x1 minute in 2.2 dimethoxypropane (DMP) which solubilized the iodoantipyrine completely without removing deoxyglucose (Diemer and Rosenørn 1981; Gjedde and Diemer 1985; Owman and Diemer 1985). These sections were placed on ^3H sensitive film (LKB Ultrofilm). The exposure time was 19 days. The neighboring sections were placed on an X-ray film (Kodak MIN-R) with a protective gelatin coating which absorbed the ^3H -radiation without affecting the ^{14}C radiation. No penetration of the coating by the ^3H -radiation was found with this exposure time even when the amount of ^3H -deoxyglucose injected was increased three times (to be published). Two sets of standards were used; one calibrated for tissue ^{14}C content, and one for ^3H content in DMP treated sections. The optical density of the same brain

areas on the autoradiograms was measured using the Leitz TAS Plus image analyzer and converted to ^{14}C or ^3H activity.

The CBF was calculated from the brain tissue ^{14}C activity determined by autoradiography using the equation of Gjedde et al. (1980):

$$f^{bl} = \frac{C_{Br}(T)}{E(T) \int_0^T C_a(t) dt}$$

where f^{bl} is the blood flow per unit mass, $C_{Br}(T)$ the isotope content, $E(T)$ the net extraction fraction of the isotope in the time from $t=0$ to $t=T$. t = the variable time and T = the experimental time (20 seconds). $C_a(t)$ is the arterial blood concentration of the isotope at time t .

The CMRglu was calculated from the plasma deoxyglucose integral, the plasma glucose concentration and the ^{14}C -activity in the brain tissue according to Sokoloff et al. (1977). The value used for the lumped constant in pre SAH animals was that determined by Sokoloff et al. (1977) (0.483 ± 0.107 ; mean $\pm$ SD). The lumped constant used for calculating the CMRglu in the low flow areas in the SAH animals was that calculated in a previous communication (Delgado et al. 1986) and found to be 0.61 ± 0.03 (mean $\pm$ SD). Outside the low flow areas it was 0.48 ± 0.07 (mean $\pm$ SD).

EXPERIMENTAL DESIGN.

The studies were carried out between two and four weeks after the stereotactic lesions.

I. LESIONS OF THE ASCENDING CA PATHWAYS IN THE MESENCEPHALON.

LCBF and LCMRglu studies were performed in five animals not subjected to cisternal blood injection and in five animals two days after a SAH.

II. LESIONS OF THE ASCENDING CA PATHWAYS IN THE MEDULLA OBLONGATA. LCBF and LCMRglu studies were carried out in five animals not subjected to cisternal blood injection and in five animals two days after a SAH.

III.SHAM LESIONS. LCBF and LCMRglu studies were performed in four sham lesioned animals not subjected to cisternal blood injection. Two of the animals had sham mesencephalic and two had sham medullary lesions. Four similarly sham lesioned animals were examined two days after a cisternal blood injection.

STATISTICAL ANALYSIS

The data were evaluated with the student t-test for two group comparisons and with the Bonferroni t-test for multiple comparisons.

RESULTS

The physiological parameters are shown in Table I. There was no significant change in MABP between the experimental groups. The temperature was kept close to 37.0 C°. The pH was about 7.37, paO_2 around 130 mmHg and paCO_2 close to 37 mmHg.

The LCBF values in the sham lesioned animals were about 10% lower than the values obtained in normal animals (Table II). The decrease was significant ($p < 0.05$) in the frontal cortex. The LCMRglu values in the sham lesioned animals were similar to the values seen in normal animals. The values for the normal animals are taken from a previous communication (Delgado et al. 1986).

The CBF and the CMRglu values in animals with mesencephalic or medullary lesions were similar to the values seen in sham lesioned animals (Table II and III). Also, the values in the two lesioned groups were comparable.

Two days after a SAH, there was a decrease in CBF of about 25% and an increase in deoxyglucose uptake of about 30% in the sham lesioned animals as compared to baseline values (Table IV). The changes in CBF and CMRglu were significant in 12 of the 17 regions examined. In one of the four sham lesioned animals, there were foci where the flow was reduced to 30% and the deoxyglucose uptake increased to about 250% of control. The foci were seen in most cortical regions, in caudate-putamen and thalamus. In contrast, in the animals with mesencephalic and medullary lesions subjected to a SAH, the global CBF and the CMRglu values were similar to the baseline values and none of the animals developed focal changes (Table V).

TABLE I
Physiological parameters in the experimental groups

experimental groups	MABP (mmHg)	Hct	glucose (mmol/l)	temp °C	ph	PaO ₂ (mmHg)	PaCO ₂ (mmHg)	n
MESENCEPHALIC LESIONS	control	115±2	45±1	13.1±2.0	37.1±0.1	158±7	37.4±1.6	5
	SAH	117±10	47±1	11.6±0.7	36.8±0.1	119±15	36.7±1.3	5
MEDULLARY LESIONS	control	107±6	45±2	9.5±0.7	37.1±0.2	134±7	38.8±0.6	5
	SAH	110±9	44±2	10.3±0.2	37.3±0.2	136±4	36.6±1.2	5
SHAM LESIONS	control	118±5	44±2	9.9±1.0	36.8±0.2	134±9	37.5±1.0	4
	SAH	104±10	46±2	11.4±2.2	37.1±0.1	142±12	39.7±0.6	4

values are means ± S.E.M.

MABP = mean arterial blood pressure

Table II

Local cerebral blood flow (LCBF) and glucose metabolism (LCMRglu)
in anaesthetized, paralyzed normal⁺ or sham lesioned rats

Region	LCBF (ml/min x 100g)		LCMRglu (umol/min x 100g)	
	normal	sham lesioned	normal	sham lesioned
frontal cortex	151 $\pm$ 4	124 $\pm$ 7*	64 $\pm$ 4	59 $\pm$ 6
septum	116 $\pm$ 12	114 $\pm$ 14	48 $\pm$ 5	52 $\pm$ 4
hypothalamus	114 $\pm$ 5	101 $\pm$ 7	38 $\pm$ 3	44 $\pm$ 4
corpus callosum	51 $\pm$ 4	48 $\pm$ 5	22 $\pm$ 5	25 $\pm$ 1
caudate-putamen	160 $\pm$ 4	144 $\pm$ 9	70 $\pm$ 4	68 $\pm$ 5
parietal cortex	139 $\pm$ 12	128 $\pm$ 14	58 $\pm$ 3	59 $\pm$ 6
hippocampus	96 $\pm$ 6	85 $\pm$ 7	45 $\pm$ 3	51 $\pm$ 4
thalamus	177 $\pm$ 10	164 $\pm$ 17	59 $\pm$ 3	63 $\pm$ 5
occipital cortex	138 $\pm$ 15	116 $\pm$ 13	56 $\pm$ 5	56 $\pm$ 5
superior colliculus	146 $\pm$ 8	136 $\pm$ 10	56 $\pm$ 1	59 $\pm$ 6
inferior colliculus	184 $\pm$ 3	176 $\pm$ 10	64 $\pm$ 5	61 $\pm$ 6
cerebellar cortex	96 $\pm$ 11	84 $\pm$ 9	36 $\pm$ 2	38 $\pm$ 3
dentate nucleus	154 $\pm$ 17	132 $\pm$ 13	46 $\pm$ 7	44 $\pm$ 3
vestibular nucleus	159 $\pm$ 12	149 $\pm$ 8	49 $\pm$ 2	53 $\pm$ 4
cochlear nucleus	152 $\pm$ 7	141 $\pm$ 18	47 $\pm$ 3	42 $\pm$ 5
trigeminal nucleus	150 $\pm$ 3	143 $\pm$ 12	42 $\pm$ 3	43 $\pm$ 4
facial nucleus	154 $\pm$ 15	167 $\pm$ 4	39 $\pm$ 4	44 $\pm$ 2
	N=4	N=4	N=4	N=4

values are means $\pm$ S.E.M.

*p<0.05, **p<0.01, ***p<0.001

⁺the values for normal animals are taken from a
previous communication (Delgado et al. 1986)

Table III

Local cerebral blood flow (LCBF) and glucose metabolism (LCMRglu) in anaesthetized, paralyzed rats with mesencephalic or medullary lesions

Region	LCBF (ml/min x 100g)		LCMRglu (umol/min x 100g)	
	mesencephalic lesions	medullary lesions	mesencephalic lesions	medullary lesions
frontal cortex	122 $\pm$ 8	123 $\pm$ 6	55 $\pm$ 6	51 $\pm$ 5
septum	106 $\pm$ 9	91 $\pm$ 7	45 $\pm$ 5	41 $\pm$ 5
hypothalamus	105 $\pm$ 8	95 $\pm$ 5	44 $\pm$ 4	38 $\pm$ 4
corpus callosum	50 $\pm$ 6	46 $\pm$ 2	21 $\pm$ 4	25 $\pm$ 3
caudate-putamen	126 $\pm$ 8	130 $\pm$ 10	64 $\pm$ 7	67 $\pm$ 10
parietal cortex	139 $\pm$ 13	119 $\pm$ 7	63 $\pm$ 3	60 $\pm$ 5
hippocampus	91 $\pm$ 8	85 $\pm$ 9	52 $\pm$ 5	55 $\pm$ 4
thalamus	152 $\pm$ 5	137 $\pm$ 15	69 $\pm$ 12	58 $\pm$ 7
occipital cortex	108 $\pm$ 13	118 $\pm$ 11	52 $\pm$ 1	53 $\pm$ 4
superior colliculus	132 $\pm$ 11	125 $\pm$ 11	57 $\pm$ 6	52 $\pm$ 4
inferior colliculus	152 $\pm$ 9	147 $\pm$ 12	55 $\pm$ 4	52 $\pm$ 5
cerebellar cortex	92 $\pm$ 8	95 $\pm$ 14	39 $\pm$ 4	34 $\pm$ 2
dentate nucleus	132 $\pm$ 14	133 $\pm$ 7	40 $\pm$ 7	41 $\pm$ 3
vestibular nucleus	158 $\pm$ 17	156 $\pm$ 16	50 $\pm$ 5	50 $\pm$ 4
cochlear nucleus	121 $\pm$ 15	124 $\pm$ 7	42 $\pm$ 3	41 $\pm$ 6
trigeminal nucleus	132 $\pm$ 11	141 $\pm$ 26	46 $\pm$ 5	40 $\pm$ 5
facial nucleus	148 $\pm$ 9	147 $\pm$ 19	48 $\pm$ 8	39 $\pm$ 5
	N=5	N=5	N=5	N=5

values are means $\pm$ S.E.M.

Table IV

Local cerebral blood flow (LCBF) and glucose metabolism (LCMRglu) two days after cisternal blood injection in anaesthetized, paralyzed rats with sham lesions

Region	LCBF (ml/min x 100g)	LCMRglu (umol/min x 100g)
frontal cortex	87 $\pm$ 11 (70%)*	80 $\pm$ 5 (136%)*
septum	72 $\pm$ 5 (63%)*	60 $\pm$ 4 (115%)n.s.
hypothalamus	71 $\pm$ 4 (70%)**	55 $\pm$ 2 (125%)*
corpus callosum	39 $\pm$ 3 (81%)n.s.	32 $\pm$ 3 (128%)*
caudate-putamen	100 $\pm$ 8 (69%)**	83 $\pm$ 3 (122%)*
parietal cortex	92 $\pm$ 17 (72%)n.s.	78 $\pm$ 3 (132%)*
hippocampus	69 $\pm$ 3 (81%)*	65 $\pm$ 3 (122%)n.s.
thalamus	110 $\pm$ 14 (67%)*	81 $\pm$ 4 (129%)*
occipital cortex	98 $\pm$ 18 (85%)n.s.	75 $\pm$ 4 (134%)*
superior colliculus	101 $\pm$ 4 (74%)**	68 $\pm$ 3 (115%)n.s.
inferior colliculus	137 $\pm$ 14 (78%)*	80 $\pm$ 6 (131%)*
cerebellar cortex	67 $\pm$ 11 (80%)n.s.	51 $\pm$ 15 (134%)n.s.
dentate nucleus	96 $\pm$ 19 (73%)n.s.	62 $\pm$ 12 (141%)n.s.
vestibular nucleus	91 $\pm$ 6 (61%)**	78 $\pm$ 9 (147%)*
cochlear nucleus	93 $\pm$ 13 (66%)*	81 $\pm$ 4 (193%)*
trigeminal nucleus	96 $\pm$ 17 (67%)*	72 $\pm$ 9 (167%)*
facial nucleus	123 $\pm$ 8 (74%)**	63 $\pm$ 7 (143%)*
	N=4	N=4

values are means $\pm$ S.E.M.

()% of pre SAH values in sham lesioned animals

*p<0.05, **p<0.01, ***p<0.001

Table V

Local cerebral blood flow (LCBF) and glucose metabolism (LCMRglu) two days after cisternal blood injection in anaesthetized, paralyzed rats with mesencephalic or medullary lesions

Regions	Mesencephalic lesions			Medullary lesions		
	LCBF(ml/min x 100g)	LCMRglu(umol/min x 100g)	LCBF(ml/min x 100g)	LCMRglu(umol/min x 100g)	LCBF(ml/min x 100g)	LCMRglu(umol/min x 100g)
frontal cortex	121±6 (99%)	57±4 (104%)	130±17(106%)	58±7 (114%)	130±17(106%)	58±7 (114%)
septum	105±7 (99%)	47±4 (104%)	105±11(115%)	49±7 (120%)	105±11(115%)	49±7 (120%)
hypothalamus	106±7 (101%)	44±2 (100%)	107±15(113%)	45±7 (118%)	107±15(113%)	45±7 (118%)
corpus callosum	47±2 (94%)	20±6 (95%)	48±3 (104%)	28±2 (112%)	48±3 (104%)	28±2 (112%)
caudate-putamen	136±8 (108%)	70±2 (109%)	132±9 (102%)	69±10(103%)	132±9 (102%)	69±10(103%)
parietal cortex	139±16(100%)	66±6 (105%)	137±12(115%)	56±7 (93%)	137±12(115%)	56±7 (93%)
hippocampus	94±11(103%)	54±3 (104%)	97±15(114%)	51±6 (93%)	97±15(114%)	51±6 (93%)
thalamus	147±22(97%)	69±13(100%)	148±15(108%)	61±10(105%)	148±15(108%)	61±10(105%)
occipital cortex	114±8 (106%)	56±3 (108%)	122±12(103%)	55±8 (104%)	122±12(103%)	55±8 (104%)
superior colliculus	130±10(99%)	54±5 (95%)	125±11(100%)	50±5 (96%)	125±11(100%)	50±5 (96%)
inferior colliculus	154±12(101%)	54±4 (98%)	157±15(107%)	53±6 (102%)	157±15(107%)	53±6 (102%)
cerebellar cortex	81±11(88%)	32±4 (82%)	106±16(112%)	35±2 (103%)	106±16(112%)	35±2 (103%)
dentate nucleus	138±13(105%)	44±7 (110%)	137±11(103%)	44±4 (107%)	137±11(103%)	44±4 (107%)
vestibular nucleus	154±24(98%)	51±5 (102%)	163±14(105%)	57±11(114%)	163±14(105%)	57±11(114%)
cochlear nucleus	136±16(112%)	42±6 (100%)	136±14(110%)	44±8 (107%)	136±14(110%)	44±8 (107%)
trigeminal nucleus	118±12(89%)	41±4 (89%)	129±16(92%)	41±4 (103%)	129±16(92%)	41±4 (103%)
facial nucleus	145±21(98%)	46±2 (96%)	166±20(113%)	44±4 (113%)	166±20(113%)	44±4 (113%)
	N=5	N=5	N=5	N=5	N=5	N=5

values are means ± S.E.M.

() % of pre-SAH values in animals with mesencephalic or medullary lesions

The sham lesioned animals were drowsy and had reduced food and water intake after the SAH. None of the animals exhibited neurological deficits. The lesioned animals seemed unaffected by the SAH.

DISCUSSION

The present study has demonstrated, that lesioning of the ascending CA pathways in the mesencephalon or medulla oblongata prior to a cisternal blood injection, completely prevents the development of the global changes in CBF and CMRglu seen in normal animals or sham lesioned animals with a SAH (Delgado et al. 1986). Also, none of the lesioned animals developed focal changes in flow or metabolism. Normal animals have a global decrease in CBF of about 20% together with an increased uptake of deoxyglucose of about 30% at two days post SAH. About one-third also have foci with markedly decreased flow (about 30% of control) and increased deoxyglucose uptake (about 250% of control). Similar results were found in the sham lesioned animals in this study.

The lesions were produced by 6-OHDA. This compound has a selective destructive effect on CA axons, leaving neurons containing other transmitters intact (Tranzer and Thoenen 1967; Ungerstedt 1971). The mesencephalic lesion destroys ascending CA fibres from the locus coeruleus and the A₁ and A₂ nuclei. The locus coeruleus projects mainly to the cortex, hippocampus and cerebellum and to a minor extent to the hypothalamus (Amaral and Sinnamon 1977), while the A₁ and A₂ nuclei project predominantly to the hypothalamus (Lindvall and Björklund 1983). Lesioning of the ascending CA pathways in the mesencephalon has been found to cause a reduction in the NA content of the frontal cortex of about 95% (Ingvar et al. 1983; Blomqvist et al. 1984; Svendgaard et al. 1985), and a reduction in the diencephalon of 87% (Svendgaard et al. 1985). Only animals with at least a 95% reduction of NA in the frontal cortex were included in the present study. In a previous communication (Svendgaard et al. 1985), lesioning of the ascending CA pathways in the medulla oblongata was shown not to cause a significant reduction of NA of the frontal cortex, while it produced a decrease in NA of about 66% in the diencephalon. Animals with medullary lesions included in the present study had no significant reduction in the NA content of the frontal cortex.

In the animals with mesencephalic or medullary lesions, the CBF and CMRglu values were similar to those obtained in the sham lesioned animals. The minor general reduction in CBF seen in the sham lesioned animals as compared to normal animals could be due to the nonspecific neurotoxic effect of the ascorbic acid contained in the solvent for 6-OHDA (Hill et al. 1973; Jonsson et al. 1974).

There are several investigations evaluating the effect of mesencephalic or locus coeruleus lesions on CBF or glucose metabolism. Norberg et al. (1978) using ^{14}C ethanol in a tissue uptake technique, Dahlgren et al. (1981) using a $^{133}\text{Xenon}$ wash-out method, and Blomqvist et al. (1984) using an autoradiographic technique found that mesencephalic or locus coeruleus lesions in the rat did not cause a significant change in CBF. Bates et al. (1977) found an increase in CBF in cats following bilateral electrolytic lesions of the locus coeruleus. The conflicting data might be secondary to species differences, to difficulties in lesioning the locus coeruleus in the cat where the distribution of the locus coeruleus cells is widespread (Amaral and Sinnamon 1977), or due to the nonspecificity of electrolytic lesions. Schwartz (1978) and Savaki et al. (1983) found that locus coeruleus lesions did not significantly change local glucose metabolism in most brain regions. There are no previous studies investigating CBF or CMRglu after selective lesions of the ascending CA fibres from the A_1 and A_2 nuclei in the medulla oblongata.

The absence of changes in CBF and CMRglu in the lesioned animals two days after a cisternal blood injection is in agreement with our earlier data showing that mesencephalic or medullary lesions prior to a SAH prevent the development of angiographically visible cerebral vasospasm (Svendgaard et al. 1985). It was suggested that A_1 and/or A_2 are essential for the development of vasospasm post SAH via their projections to the hypothalamus-pituitary. The present communication indicates that these ascending CA projections also are essential for the development of the CBF and CMRglu changes seen after a SAH. However, the mechanism by which the A_1 and/or A_2 are involved in the changes of flow and metabolism after a SAH is unknown.

Blood in the subarachnoid space could affect sensory nerves on the cerebral vessels or brain tissue. An innervation of cerebral arteries from the trigeminal nerve (Mayberg et al. 1984; Arbab et al. 1986) and from the first two spinal ganglia (Arbab et al. 1986) has been

described. The nucleus tractus solitarius (NTS) which contains A_2 neurons and is a main center for afferent visceral information, is known to receive afferents from the trigeminal ganglion (Palkovits and Zaborszky 1977; Jacquin et al. 1982) and also from the second spinal ganglion (unpublished observation). The afferent information might be relayed via A_2 neurons and/or the A_1 nucleus which are closely connected to NTS (Loewy et al. 1981), to effector neurons in different brain regions. The effector neurons could be located in the parabrachial complex, the raphe nuclei, or the hypothalamus.

The A_1 and A_2 nuclei are known to project to the parabrachial complex (Ricardo and Koh 1978; Loewy et al. 1981) which can influence cerebral flow and metabolism (Reis et al. 1985). The parabrachial nucleus in turn projects to widespread brain regions including hypothalamus and amygdala (Saper and Loewy 1980). A_1 and A_2 also project to the raphe nuclei (Levitt and Moore 1979). Raphe neurons not only project to hypothalamus (Azmitia and Segal 1978; Steinbusch and Nieuwenhuys 1981), but probably also innervate the cerebral vessels (Edvinsson et al. 1983; Griffith and Burnstock 1983). It is unlikely that the serotonergic projections from the raphe nuclei are involved in the CBF and metabolic changes after a SAH, since animals with chemical lesions of the central serotonergic systems subjected to a SAH still develop flow and metabolic changes (unpublished observations). A_1 and A_2 are known to project heavily to multiple hypothalamic nuclei (Ricardo and Koh 1978; Lindvall and Björklund 1983) including the paraventricular and the supraoptic nuclei (Sawchenko and Swanson 1981). The hypothalamus as the center for coordination of autonomic and endocrine responses, might be the center involved in the CBF and metabolic changes seen post SAH.

In conclusion: Our data indicate that blood in the subarachnoid space via a neural mechanism induces not only the vasospasm but also the changes in flow and metabolism seen after a SAH. Our hypothesis is that different components of blood at different time points after the SAH could trigger the relay of information to neuronal subgroups within NTS or A_2 that project to various target neurons allowing a differentiated response concerning spasm, flow and metabolism.

ACKNOWLEDGEMENT

Supported by grants from the Einar Björkelund Foundation; Anna-Lisa and Sven-Eric Lundgren Foundation; Torsten and Elsa Segerfalk Foundation; Danish Medical Research Council (14X-732); Medical Faculty, University of Lund and the Swedish Medical Research Council (NO 04X-5300-05 and 14X-732)

REFERENCES

- Amaral DG, Sinnamon HM (1977) The locus coeruleus: neurobiology of a central noradrenergic nucleus. *Progress in Neurobiology* 9:147-196
- Arbab MAR, Wiklund L, Svendgaard N Aa (1986) Origin and distribution of the cerebral vascular innervation from superior cervical, trigeminal and spinal ganglia investigated with retrograde and anterograde WGA-HRP tracing in the rat. *Neuroscience* 0:00
- Azmitia EC, Segal M (1978) An autoradiographic analysis of the differential ascending projections of the dorsal and median raphe nuclei in the rat. *J Comp Neurol* 179:641-668
- Bates D, Weinshilboum RM, Campbell RJ, Sundt TM Jr. (1977) The effect of lesions in the locus coeruleus on the physiological responses of the cerebral blood vessels in cats. *Brain Res* 136:431-443
- Blomqvist P, Lindvall O, Wieloch T (1984) Delayed postischemic hypoperfusion: Evidence against involvement of the noradrenergic locus ceruleus system. *J Cereb Blood Flow Metabol* 4:425-429
- Dahlgren N, Lindvall O, Sakabe T, Siesjö BK (1981) Cerebral blood flow and oxygen consumption in the rat brain after lesions of the noradrenergic locus coeruleus system. *Brain Res.* 209:11-23
- Dahlström A, Fuxe K (1964) Evidence for the existence of monoamine-containing neurons in the central system. I. Demonstration of monoamines in the cell bodies of brain stem neurons. *Acta Physiol Scand* 62 (Suppl.232):1-55
- Delgado TJ, Arbab MAR, Diemer NH, Svendgaard N Aa (1986) Subarachnoid haemorrhage in the rat: cerebral blood flow and glucose metabolism during the late phase of cerebral vasospasm. Submitted to *J Cereb Blood Flow Metabol*
- Delgado TJ, Brismar J, Svendgaard N Aa (1985) Subarachnoid haemorrhage in the rat: angiography and fluorescence microscopy of the major cerebral arteries. *Stroke* 16:595-602
- Diemer NH, Rosenørn J (1981) Determination of local cerebral blood flow and glucose metabolism or transfer by means of a double autoradiographic method. *J Cereb Blood Flow Metabol* 1:S72-S73
- Edvinsson L, Degueurce A, Duverger D, MacKenzie ET, Scatton B (1983) Central serotonergic nerves project to the pial vessels of the brain. *Nature* 306:55-57
- Eriksson B-M, Persson B-A (1982) Determination of catecholamines in rat heart tissue and plasma samples by liquid chromatography with electrochemical detection. *J Chromatography* 228:143-154

- Gjedde A, Diemer NH (1985) Double-tracer study of the fine regional blood-brain glucose transfer in the rat by computer-assisted autoradiography. *J Cereb Blood Flow Metabol* 5:282-289
- Gjedde A, Hansen AJ, Siemkowicz E (1980) Rapid simultaneous determination of regional blood flow and blood-brain glucose transfer in brain of rat. *Acta Physiol Scand* 108:321-330
- Griffith SG, Burnstock G (1983) Immunohistochemical demonstration of serotonin in nerves supplying human cerebral and mesenteric blood-vessels. *Lancet* March 12:561-562
- Grubb RL, Raichle ME, Eichling JO, Gado MH (1977) Effects of subarachnoid hemorrhage on cerebral blood volume, blood flow, and oxygen utilization in humans. *J Neurosurg* 46:446-453
- Hill CE, Mark GE, Eränkö O, Eränkö L, Burnstock G (1973) Use of tissue culture to examine the actions of guanethidine and 6-hydroxydopamine. *Eur J Pharmacol* 23:162-174
- Iadecola C, Nakai M, Mraovitch S, Ruggiero DA, Tucker LW, Reis DJ (1983) Global increase in cerebral metabolism and blood flow produced by focal electrical stimulation of dorsal medullary reticular formation in rat. *Brain Res* 272:101-114
- Ingvar DH, Söderberg U (1958) Cortical blood flow related to EEG patterns evoked by stimulation of brain stem. *Acta Physiol Scand* 42:130-143
- Ingvar M, Lindvall O, Folbergrova J, Siesjö BK (1983) Influence of lesions of the noradrenergic locus coeruleus system on the cerebral metabolic response to bicuculline-induced seizures. *Brain Res* 264:225-231
- Jacquin MF, Semba K, Rhoades RW, Egger MD (1982) Trigeminal primary afferents project bilaterally to dorsal horn and ipsilaterally to cerebellum, reticular formation, and cuneate, solitary, supratrigeminal and vagal nuclei. *Brain Res* 246:285-291
- Jonsson G, Lohmander S, Sachs Ch (1974) 6-Hydroxydopamine induced degeneration of noradrenaline neurons in the scorbutic guinea-pig. *Biochem Pharmacol* 23:2585-2593
- LeBeau J, Rabinovitch R (1949) Les voies hypothalamiques descendantes et la régulation vasomotrice du cerveau chez l'homme et chez l'animal. *Rev Canad de Biol* 8:430-438
- Levitt P, Moore RY (1979) Origin and organization of brainstem catecholamine innervation in the rat. *J Comp Neurol* 186:505-528
- Lindvall O, Björklund A (1983) Dopamine- and norepinephrine-containing neuron systems: their anatomy in the rat brain. In: *Chemical Neuroanatomy* (ed ICP Emson) New York, Raven Press, pp.229-255
- Loewy AD, Wallach JH, McKellar S (1981) Efferent connections of the ventral medulla oblongata in the rat. *Brain Res Rev* 3:63-80
- Luis J (1879) *Le cerveau et ses fonctions*. Paris/Germer-Baillier & Cie
- Mayberg MR, Zervas NT, Moskowitz MA (1984) Trigeminal projections to supratentorial pial and dural blood vessels in cats demonstrated by horseradish peroxidase histochemistry. *J Comp Neurol* 223:46-56
- Mraovitch S, Iadecola C, Reis DJ (1983) Vasoconstriction unassociated with

- metabolism in cerebral cortex elicited by electrical stimulation of the parabrachial nucleus in rat. *J Cereb Blood Flow Metabol* 3 (Suppl 1):S196-197
- Norberg K, Scatton B, Korf J (1978) Amphetamine-induced increase in rat cerebral blood flow: apparent lack of catecholamine involvement. *Brain Res* 149:165-174
- Owman Ch, Diemer NH (1985) Studies of LCBF and glucose metabolism in brain by computer assisted autoradiography. In *Quantitative Neuroanatomy in Transmitter Research* (eds K Fuxe, LF Agnati) London, MacMillan Press, pp. 71-87
- Palkovits M, Zaborszky L (1977) Neuroanatomy of central cardiovascular control. Nucleus tractus solitarii: afferent and efferent neuronal connections in relation to the baroreceptor arc. *Prog Brain Res* 47:9-34
- Pool JL (1958) Cerebral vasospasm. *N Eng J Med* 259:1259-1264
- Reis DJ, Iadecola C, Nakai M (1985) Control of cerebral blood flow and metabolism by intrinsic neural systems in brain. In *Cerebrovascular Diseases* (eds F. Plum, W. Pulsinelli), New York, Raven Press, pp 1-22
- Reivich M, Jehle J, Sokoloff L, Kety SS (1969) Measurement of regional cerebral flow with antipyrine- ^{14}C in awake rats. *J Appl Physiol* 27:296-300
- Ricardo JA, Koh ET (1978) Anatomical evidence of direct projections from the nucleus of the solitary tract to the hypothalamus, amygdala, and other forebrain structures in the rat. *Brain Res* 153:1-26
- Sahlin Ch, Brismar J, Delgado TJ, Owman Ch, Salford LG, Svendgaard NAA (1986) Cerebrovascular and metabolic changes during the delayed vasospasm following experimental subarachnoid hemorrhage in baboons, and treatment with a calcium antagonist. Submitted for publication
- Sakurada O, Kennedy C, Jehle J, Brown JD, Carbin GL, Sokoloff LC (1978) Measurement of local cerebral blood flow with iodo- ^{14}C antipyrine. *Am J Physiol* 234(1): H59-H66
- Saper CB, Loewy AD (1980) Efferent connections of the parabrachial nucleus in the rat. *Brain Res* 197:291-317
- Savaki HE, Graham DI, McCulloch J (1983) Differential effects of locus coeruleus lesions upon metabolic activity in CNS nuclei involved in cardiovascular regulation. *Brain Res* 271: 109-114
- Sawchenko PE, Swanson LW (1981) Central noradrenergic pathways for the integration of hypothalamic neuroendocrine and autonomic responses. *Science* 214:685-687
- Schwarz WJ (1978) 6-Hydroxydopamine lesions of rat locus coeruleus alter brain glucose consumption, as measured by the 2-deoxy-D- ^{14}C glucose tracer technique. *Neurosci Lett* 7:141-150
- Sokoloff L, Reivich M, Kennedy C, Des Rosiers MH, Patlak CS, Pettigrew KD, Sakurada O, Shinohara M (1977) The ^{14}C deoxyglucose method for the measurement of local cerebral glucose utilization: theory, procedure and normal values in the conscious and anesthetized albino rat. *J Neurochem* 28: 897-917
- Steinbusch HWM, Nieuwenhuys R (1981) Localization of serotoninlike immunoreactivity in the central nervous system and pituitary of the rat, with special references to the innervation of the hypothalamus. In *Serotonin: Current Aspects of Neurochemistry and Function* (eds B Haber, S Gabay, MR Issidorides, SGA

- Alivisatos) New York/London, Plenum Press, pp. 7-35
- Svendgaard N Aa, Brismar J, Delgado TJ, Rosengren E (1985) Subarachnoid haemorrhage in the rat: effect on the development of vasospasm of selective lesions of the catecholamine systems in the lower brain stem. *Stroke* 16:602-608
- Tranzer JP, Thoenen HC (1967) Ultramorphologische Veränderungen der Sympatischen Nervenendigungen der Katze nach Vorbehandlung mit 5- und 6-Hydroxydopamine. *Naunyn Schmiedebergs Arch Pharmak Exp Path* 257: 343-344
- Ungerstedt U (1971) Effect of 6-hydroxydopamine on the monoamine-containing neurons in the CNS. Histochemical studies on the effect of intracerebral and intraventricular injections of 6-hydroxydopamine on monoamine neurons in the rat brain. In: 6-Hydroxydopamine and Catecholamine Neurons (eds T Malmfors, H Thoenen) Amsterdam, North Holland, pp. 101-127
- Westerberg V, Lewis J, Corcoran ME (1984) Depletion of noradrenaline fails to affect kindled seizures. *Exp Neurol* 84:237-240

Effect of selective lesions in the hypothalamic-pituitary region on the development of cerebral vasospasm following an experimental subarachnoid haemorrhage in the rat.

N.Aa. Svendgaard, T.J. Delgado and *A. Brun

*Neurosurgical Research Department and *the Neuropathological Institute, University Hospital, Lund, Sweden.*

SUMMARY

Intracisternal injection of blood in the rat induces an angiographically demonstrable, biphasic cerebral vasospasm with a maximal acute spasm at ten minutes and a maximal late spasm at two days after the subarachnoid haemorrhage. Systemic administration of 6-hydroxydopamine that destroys catecholamine fibres in the circumventricular areas characterized by the absence of a blood-brain barrier, prevents the development of both the acute and the late spasm. Isolation or removal of one of the circumventricular organs, the pituitary, from the brain via a stalk transection or a hypophysectomy, does not affect the degree of vasospasm. Lesion of the median eminence, another region without a blood-brain barrier, prevents the development of both types of spasm. The median eminence receives projections from the A_1 and A_2 nuclei in the medulla oblongata. Lesioning of the ascending catecholamine pathways from these nuclei has been shown to prevent the occurrence of spasm. The A_1 and A_2 nuclei in the medulla oblongata via their projections in the central tegmental tract to the median eminence, are the intracerebral catecholamine systems underlying the development of vasospasm. The mechanism by which the median eminence exerts its vascular effect remains to be investigated.

INTRODUCTION

The theory that the hypothalamus is involved in the development of cerebral arterial vasospasm following a subarachnoid haemorrhage (SAH) has been advocated by Wilkins (1975). A role for the hypothalamus in the development of vasospasm is supported by the not infrequent occurrence of spasm following craniotomy for intra- or suprasellar tumors, while spasm does not appear following intracranial surgery not affecting the hypothalamic region (Kågström et al. 1969; Simeone and Peerless 1975; Peerless 1980). Similarly, the development of vasospasm after a skull trauma, with or without blood in the subarachnoid space, might also be due to damage of the hypothalamus (Wilkins and Odom 1970; Suwanwela and Suwanwela 1972; Macpherson and Graham 1978). Additional evidence for a hypothalamic involvement in spasm is the frequent occurrence of autonomic hyperactivity following SAH or skull trauma (Hammer-

meister and Reichenbach 1969; Offerhaus and Van Gool 1969, Hawkins and Clower 1971; Cruickshank et al. 1974; Neil-Dwyer et al. 1974; Lacy and Earle 1983). Furthermore, Wilson and Feild (1974) found that injection of a hypothalamic extract into the cisterna magna in dogs produced a prolonged cerebral vasospasm.

In previous communications (Svendgaard et al. 1985a, 1986), we have shown that ascending catecholamine (CA) pathways projecting to the hypothalamus from the medulla oblongata underlie the development of spasm following an experimental SAH. In the present study, we further evaluate the role of the hypothalamus in the development of vasospasm by applying selective lesions in the hypothalamus-pituitary region to a rat SAH model (Delgado et al. 1985).

MATERIAL AND METHODS

The experiments were performed on male Sprague-Dawley rats (SPF strain Møllegaards Avlslaboratorium, Køge, Denmark) weighing between 280 and 350 g.

ANAESTHESIA AND SURGICAL PROCEDURES

After initiation of the anaesthesia with 4% halothane, the animals were intubated and artificially ventilated. For the surgical procedure, anaesthesia was maintained with 0.75% halothane in a 70% nitrous oxide and 30% oxygen mixture. After infiltrating the skin with lidocain hydrochloride (Xylocain®, Astra), a catheter was inserted into the axillary artery bilaterally for subsequent angiography (For technical details see Delgado et al. 1985). The femoral artery and vein were cannulated for continuous blood pressure monitoring and for infusion of drugs. Heparin (Vitrum 75 IU/kg) was given intravenously. After surgery, the halothane was switched off and suxamethonium chloride (Celocurin-klorid, Vitrum 3 mg/kg i.v.) was given. Thirty minutes were allowed to pass before the angiography.

ANGIOGRAPHY

Vertebro-basilar angiography was performed via bilateral axillary catheters using metrizamide (Amipaque®, Nyegaard and Co.,

Oslo, Norway) as the contrast medium. The control angiography was followed by the injection of 0.07 ml of homologous blood intracisternally via a previously implanted catheter connected to the cisterna magna (Delgado et al. 1985). Repeat angiography was performed ten minutes and/or two days after the blood injection, the time points of maximal acute and late spasm respectively in the rat (Delgado et al. 1985). Measurements of the vertebral and basilar arteries were made using a technique similar to that described by Gabrielsen and Greitz (1970). The diameters at four preselected points within the vertebro-basilar system were averaged and expressed as percentage of control values.

LESIONS

SYSTEMIC 6-HYDROXYDOPAMINE (6-OHDA) INJECTION. 6-OHDA (100 mg/kg i.v.) was given to animals under barbiturate anaesthesia (Brietal®, Lilly, 40 mg/kg i.p.).

TRANSECTION OF THE PITUITARY STALK. The animals were anaesthetized, intubated and ventilated as described. The surgical approach was that outlined by Porter and Smith (1967) and will be presented briefly. A dissecting Zeiss microscope was used. The animals were placed in a supine position. Via a midline incision, the soft tissue was retracted until the two capitus longus muscles could be seen. The muscles were scraped off and the basisphenoid bone lying ventral to the pituitary was visualized. Using a dental drill, the pituitary and the stalk were exposed. The stalk was cut with angled scissors where it penetrated the sellular diaphragm and immediately before it joined the pituitary (Fig 1). The pituitary was removed in about half of the animals. In the remaining animals the pituitary was left in situ and oxycel cotton (Deseret Medical Inc. Parke Davis and Co., USA) was inserted into the interspace between the pituitary and the stalk end. In the sham lesioned animals, the stalk was exposed but no lesion was made.

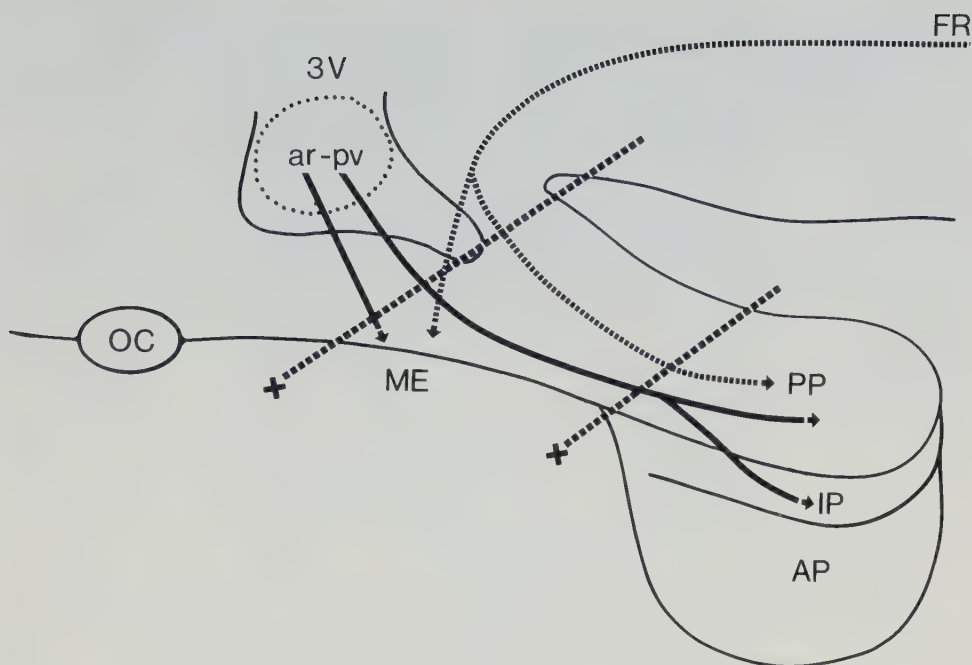


Fig 1. Midline sagittal section of rat hypothalamus and pituitary illustrating the surgical lesions of the median eminence and stalk. Broken lines indicated by stars show lesion sites. Solid lines with arrows indicate the dopaminergic innervation while broken lines with arrows show the noradrenergic innervation. ME median eminence; OC optic chiasm; FR reticular formation; 3V third ventricle; AP anterior pituitary; IP intermediate pituitary; PP posterior pituitary; ar-pv arcuate nucleus—periventricular nucleus.

MEDIAN EMINENCE LESION

The lesion was made either surgically or electrothermically using a stereotaxic technique. The approach for the surgical lesion was the same as that described for the stalk lesion. By inserting a blunt tipped hook above the horizontally oriented stalk, the anterior extension of the stalk could be determined. The hook was then replaced by an injection needle No 20 with the bevel bent 90 degrees. From the anterior part of the stalk, the needle was pushed forward about one mm until the median eminence was separated from the hypothalamus (Fig 1).

The electrothermic lesions were made using a Radionics lesion generator (RFG-4A). The diameter of the electrode was 0.25 mm. It was insulated except for the distal 0.25 mm. The animals were anaesthetized with Brietal (Lilly, 40 mg/kg i.p.) and placed in a Kopf stereotaxic frame. With the toothbar at 0, the electrode was inserted 5.1 mm anterior to the interaural line and 0.2 mm from the midline bilaterally. After lowering the electrode to the base of the skull, it was elevated 0.3 mm and the lesions produced by raising the temperature of the electrode tip to 67 °C for one minute (Fig 2B). In the sham lesioned animals, the electrode was lowered to the skull base and then withdrawn.

CONTROL OF THE LESIONS.

The lesion produced by intravenous injection of 6-OHDA was controlled by fluorescence microscopy of the irides. 6-OHDA (100 mg/kg i.v.) produces a chemical sympathectomy which in the iris lasts about three weeks (Malmfors and Sachs 1968).

The animals with distal stalk transections with or without hypophysectomies were perfused via the axillary artery catheters with 1% glutaraldehyde and 2% formaldehyde. After decapitation, the soft tissue and bone covering the dorsal surface of the brain were removed before storing the specimen in the glutaraldehyde-formaldehyde solution at 0°C. In half of the animals, the brain was removed from the skull before sectioning, while in the other half it was kept in situ with the skull bone intact for sectioning. The brain was cut in 20 µm sections and stained with haematoxylin-eosin. Microscopy revealed that about 0.5 mm of the proximal part of the stalk was left intact and that it contained cells of normal appearance. The basal hypothalamus was undamaged.

The animals with median eminence lesions were processed similarly to the stalk lesioned animals. The length of the electrothermic lesion was close to 1.4 mm and included 0.4-0.5 mm of the proximal part of the stalk. The width was about 1 mm and the height at its maximum was also about 1 mm (Fig 2B). The lesion extended into the arcuate nuclei. In the animals with surgical lesions, no median eminence tissue was seen, and the damage to the arcuate nuclei was negligible.

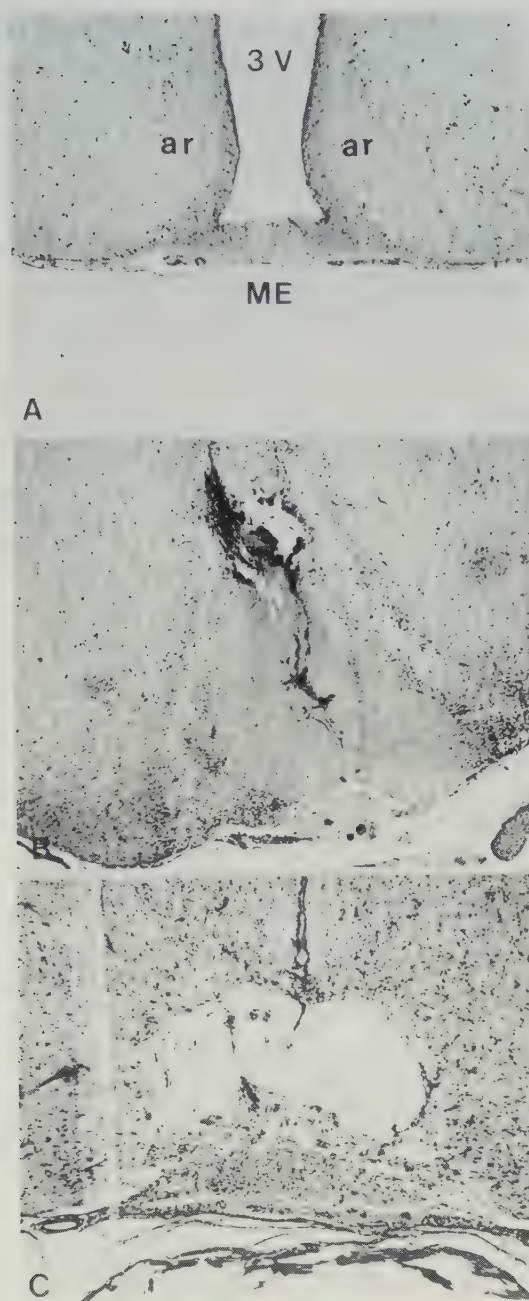


Fig 2. A-C. Light photomicrographs (haematoxylin-eosin) of coronal sections of rat brain through the median eminence. A. Median eminence from a normal rat. B. Complete electrothermic lesion of the median eminence. C. Incorrectly placed electrothermic lesion affecting the arcuate nucleus but largely sparing the median eminence. Magnification $\times 40$. ar = arcuate nucleus. ME = median eminence. 3 V = third ventricle.

In a separate group of six animals, the plasma prolactin level was examined before and 14 days after electrothermic median eminence lesions and showed an increase of about 70% in the lesioned animals.

STATISTICS

The data were analyzed using the Student t-test.

EXPERIMENTAL DESIGN

There were six experimental groups. The normal animals were taken from an earlier study (Delgado et al. 1985). The lesions were made 9-14 days prior to the angiography. The angiography was performed ten minutes and/or two days after the cisternal blood injection. The animals with median eminence, stalk or sham lesions received hydrocortisone (Solu-Cortef®, Upjohn; 2 mg i.v.) the day before and the day of implantation of the subcutaneous catheter. The animals also had hydrocortisone (2 mg) the day before angiography and each subsequent day until sacrifice.

Group I. Six normal animals.

Group II. Six animals treated with 6-OHDA i.v.

Group III. Four normal animals treated with hydrocortisone according to the schedule used for the lesioned animals.

Group IV. Eleven animals with pituitary stalk lesions. Six animals also had the pituitary removed. Three of the eleven were examined at both ten minutes and two days. The remaining eight were examined at either ten minutes or two days post SAH.

Group V. Nine animals had median eminence lesions (three with surgical and six with electrothermic). Five were examined at both time points. The remaining four were examined at either ten minutes or two days.

Group VI. Three animals with sham median eminence and three with sham pituitary stalk lesions. The values from these animals were pooled.

RESULTS

The physiological parameters in the different experimental groups are shown in Table I. All animals had rectal temperatures close to 37°C. PaO₂ was about 140 mmHg. PaCO₂ was about 37 mmHg. The pH was close to 7.4. There were no significant differences in the MABP between the experimental groups.

In the four normal animals treated with steroids, the degree of spasm post SAH was similar to that seen in normal SAH animals (mean vessel diameter in percent of control $\pm$ SEM: at ten minutes 68.3 $\pm$ 6.6 for the steroid treated group versus 66.4 $\pm$ 4.8 for the normal animals; at two days 76.5 $\pm$ 3.5 for the steroid treated group versus 77.5 $\pm$ 6.0 for the normal animals). Also, the degree of spasm in the sham lesioned animals was similar to that seen in normal animals.

Intravenous 6-OHDA treatment prior to cisternal blood injection prevented the development of vasospasm at both ten minutes and two days post SAH (Fig 3). The baseline mean vessel diameter in the vertebro-basilar system was similar to that seen in normal animals.

The animals with stalk lesions had a similar degree of spasm as the sham lesioned animals (Fig 3 and Fig 4). There was no difference in the degree of spasm in the animals with or without the pituitary removed. Sectioning of the stalk did not change the baseline mean vessel diameter.

Lesion of the median eminence prevented the development of both acute and late spasm (Fig 3 and Fig 5). There was a minor dilatation of the vertebro-basilar arteries at both ten minutes and two days post SAH. The baseline diameter was unchanged. There was no difference in the angiographical findings between the surgically or the electrothermically lesioned animals. In an additional two animals not included in the study, the electrothermic lesions were placed 0.5 mm instead of 0.3 mm above the base of the skull. Both animals developed acute and late spasm similar that seen in the sham lesioned group. The light microscopical examination of the hypothalamus in these two animals showed no change in the median eminence except for a slight edema in the internal layer (Fig 2C). The lesions were localized to the arcuate nuclei.

TABLE I

Physiological parameters pre and post SAH

	MAP mmHg	pulse	pH	PaO ₂	PaCO ₂	N
normal	control	336±26	7.44±0.03	156±13	35.4±1.6	6
	10 min	285±15	7.44±0.03	156±13	35.4±1.6	6
	2 days	282±32	7.44±0.04	150±17	35.1±1.0	6
normal + steroids	control	315±15	7.40±0.02	143±9	37.9±1.3	4
	10 min	330±30	7.38±0.03	157±11	37.4±1.5	4
	2 days	273±33	7.41±0.04	155±10	35.5±1.8	4
sham stalk or median eminence lesions	control	306±38	7.38±0.02	144±11	36.6±0.9	6
	10 min	360±55(4)	7.37±0.02	144±13	37.2±0.8	6
	2 days	288±22	7.39±0.03	146±9	35.7±0.9	6
i.v. 6-OHDA	control	345±29	7.40±0.02	146±9	39.3±1.4	6
	10 min	294±20(5)	7.41±0.03	149±12	38.2±1.5	6
	2 days	336±24	7.38±0.03	152±24	37.5±1.3	6
stalk transections	control	286±15	7.37±0.02	139±11	36.7±1.0	11
	10 min	260±25	7.37±0.03	134±15	36.4±1.0	7
	2 days	257±17	7.37±0.01	151±7	37.6±1.3	7
median eminence lesions	control	334±22	7.38±0.01	122±9	37.2±1.0	9
	10 min	326±22	7.37±0.01	117±12	37.3±1.2	7
	2 days	312±29	7.36±0.02	152±6	37.9±1.1	7

Values are means ± S.E.M.

MAP= mean arterial blood pressure

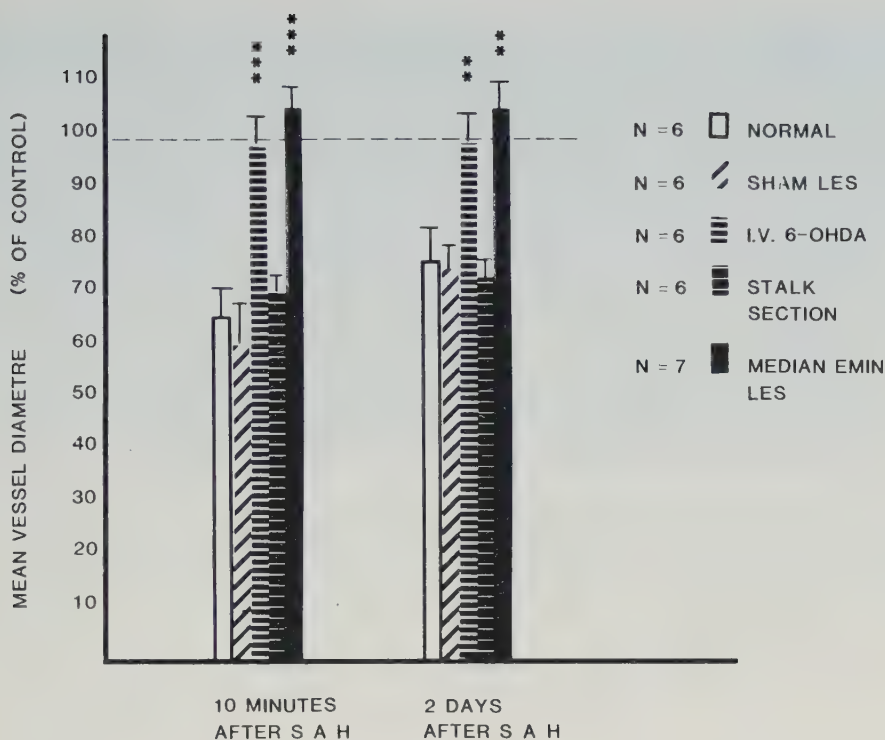


Fig 3. Angiographical changes in mean vertebo-basilar diameter post SAH after i.v. 6-OHDA, stalk transection or median eminence lesions. The values are means $\pm$ S.E.M. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

There was a rather high mortality in the animals with surgical lesions of the stalk or median eminence, and the animals included in the study represent only a part of a much larger group. The mortality in the animals with electrothermic lesions was minor, and there was no mortality in the 6-OHDA treated animals. The animals developing spasm post SAH were drowsy, but did not exhibit focal neurological deficits. The animals not developing spasm seemed unaffected by the cisternal blood injection.

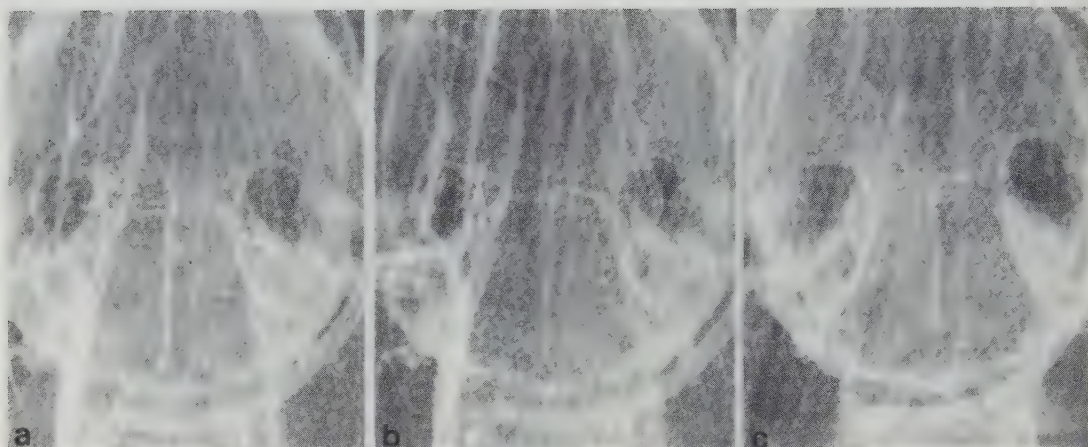


Fig 4. Vertebro-basilar angiography in an animal with transection of the pituitary stalk. A. Control. B and C. Ten minutes and two days respectively, after cisternal blood injection. Spasm is seen at both ten minutes and two days post SAH.

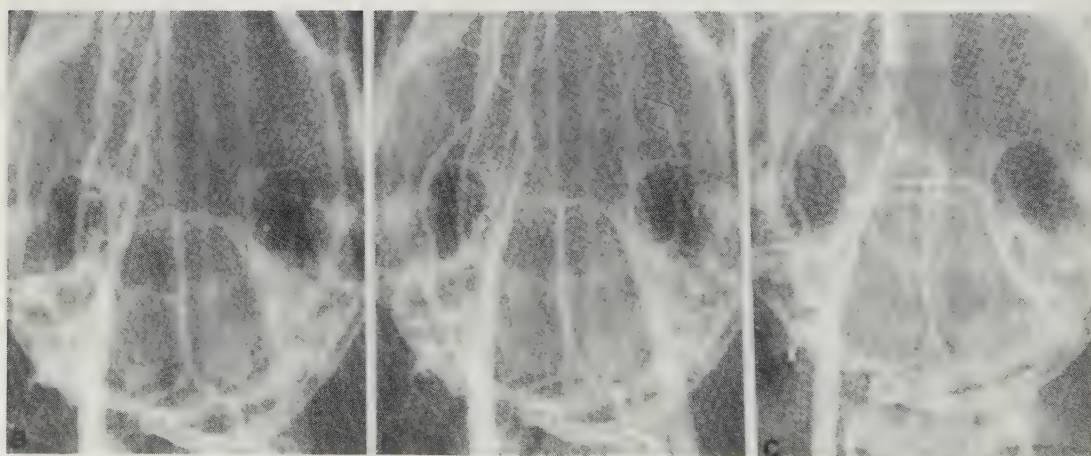


Fig 5. Vertebro-basilar angiography in an animal with lesion of the median eminence. A. Control. B and C. Ten minutes and two days respectively, after cisternal blood injection. No spasm is seen at either ten minutes or two days post SAH.

DISCUSSION

In previous communications (Svendgaard et al. 1985a, 1986), it was demonstrated that ascending CA projections from the A₁ and A₂ nuclei in the medulla oblongata to the hypothalamus or the pituitary are necessary for the development of vasospasm post SAH. In the present study, we have shown that intravenous injection of 6-OHDA or lesion of the median eminence prevents the development of vasospasm post SAH, while caudal pituitary stalk lesion does not affect either the acute or late spasm.

6-OHDA has a selective destructive effect on CA terminals and axons, leaving neurons containing other transmitters intact (Tranzer and Thoenen 1967; Sachs and Jonsson 1975). Peripherally, 6-OHDA acts on the sympathetic nervous system (Malmfors 1971). After injection of 100 mg 6-OHDA/kg i.v., the depletion of noradrenaline (NA) from the nerve terminals starts immediately and is completed within a few hours (Malmfors and Sachs 1968; de Champlain 1971). The regeneration of the adrenergic nerve terminals starts within three to four days, is extensive at three weeks and complete after two to three months (Finch et al. 1973; de Champlain et al. 1975).

Since 6-OHDA does not penetrate the blood-brain barrier (Laverty et al. 1965), the central effect of the compound is seen in areas not protected by the barrier such as the median eminence and the posterior and intermediate lobe of the pituitary (Baumgarten et al. 1972; Jonsson et al. 1972; Smith and Helme 1974; Day and Willoughby 1980; Smith et al. 1982a; Lim et al. 1984), the area postrema (Brizzee et al. 1978) and the subfornical organ (Palazzo et al. 1978). Day and Willoughby (1980) and Smith et al. (1982a) demonstrated that intravenous 6-OHDA treatment produces a complete depletion of CA in the internal layer and an almost complete CA depletion in the external layer of the median eminence within 24 hours. These authors found a substantial return of the CA fluorescence in the external layer by day seven post injection but not until day 21 was there a strong CA fluorescence in the internal layer of the median eminence.

The dopamine (DA) terminals are predominantly found in the external layer of the median eminence, while the NA terminals mainly are confined to the internal layer (Löfström et al. 1976; Day and Willoughby 1980). The DA innervation originates via the tuberoinfundibular system from the arcuate and periventricular nuclei (Jonsson et al. 1972; Björklund et al. 1973). The median eminence

has both a sympathetic innervation (Gallardo et al. 1984) and a central supply of NA terminals from the lower brain stem as shown by lesioning experiments (Jonsson et al. 1972; Löfström et al. 1976; Palkovits et al. 1977). In a retrograde tracing experiment using wheat germ agglutinin conjugated to horseradish peroxidase, we have found a monosynaptic connection between the median eminence and the A₂ nucleus (unpublished observation).

Intravenous 6-OHDA treatment also produces a depletion of the CA fibres of the intermediate and posterior lobe of the pituitary that seems to be more longlasting than that in the median eminence (Baumgarten et al. 1972; Lim et al. 1984). The intermediate and posterior lobes have a DA innervation via the tuberoinfundibular system (Björklund et al. 1970, 1973) and a NA innervation of both peripheral and central origin (Björklund et al. 1973; Alper et al. 1980; Saavedra 1985).

The stalk transection destroys both the central NA and DA innervation of the posterior and intermediate lobe of the pituitary in addition to damaging the portal veins to the anterior lobe. The sympathetic fibres from the superior cervical ganglion to the posterior lobe are not affected (Saavedra 1985).

The surgical lesion of the median eminence, in addition to transecting the stalk, also destroys the central NA and DA containing terminals and disrupts the portal system of the median eminence. The electrothermic lesion produces destruction of the same structures encompassed by the surgical lesion, but also damages the arcuate nucleus in the mediobasal hypothalamus. The increase in plasma prolactin levels seen in the present study and in previous investigations (Bishop et al. 1972; Weisenberg et al. 1979) verifies that there is a damage of the DA terminals in the median eminence since DA tonically suppresses the prolactin secretion from the anterior lobe of the pituitary (McCann et al. 1979).

The absence of spasm in the 6-OHDA treated animals is not likely to be due just to the damage to the sympathetic system. In separate experiments in animals with cranial sympathectomy prior to a SAH, we found that both acute and late spasm developed although the degree of late spasm was reduced (Svendgaard et al. 1985b). Instead, the data suggested that CA neurons projecting to a circumventricular organ were involved in the development of vasospasm. In view of our previous findings that lesioning of the ascending medul-

lary CA pathways projecting to the hypothalamus and the pituitary prevented the development of spasm (Svendgaard et al. 1985a), it was most probable that the median eminence and/or the neurointermediate lobe were the sites coresponsible for the spasm. The findings from the stalk lesioned animals show that the neurointermediate lobe of the pituitary is not participating, implying that the median eminence is the site coresponsible for vasospasm. The results further indicate that it is the NA terminals within the median eminence that are essential for the spasm development. It is unlikely that the DA fibres in the median eminence are involved since they have a high resistance to 6-OHDA (Jonsson et al. 1972; Cuello et al. 1974) probably due to the lack of a high affinity uptake system (Demarest et al. 1979). This is reflected in the rapid reappearance of the DA fluorescence in the median eminence after 6-OHDA treatment as opposed to the NA fluorescence that does not reappear until after three weeks (Day and Willoughby 1980; Smith et al. 1982a). In addition, the DA mechanisms of the median eminence seem not to be functionally impaired as shown by the normal plasma level of prolactin in 6-OHDA treated animals (Day and Willoughby 1980; Smith et al. 1982b). Furthermore, in two animals with lesions placed in the arcuate nuclei which are the source of DA innervation of the median eminence, spasm still developed to a normal extent. Finally, three weeks after the 6-OHDA treatment, which is the time point when the NA fibres reappear in the median eminence, the animals again develop spasm after a SAH (unpublished observation).

The ascending NA pathways from the medulla oblongata are suggested to relay interoceptive sensory signals to the hypothalamus for the coordination of autonomic and endocrine responses (Hansen et al. 1981; Sawchenko and Swanson 1981; Iversen 1984). The NA fibres from the lower brain stem project to the median eminence directly (Palkovits et al. 1977) and also to the hypothalamic nuclei (Lindvall and Björklund 1983) that produce neuropeptides released in or traversing the median eminence (Palkovits 1982). The neuropeptides include releasing or inhibiting hormones (luteinizing hormone releasing hormone, thyrotropin releasing hormone, somatostatin), posterior lobe hormones (vasopressin, oxytocin) and other peptides such as endorphins, enkephalins, adrenocorticotropin, melanocyte-stimulating hormone and cholecystokinin (Wood 1982).

Generally, the NA neurons that terminate in the median

eminence facilitate the release of neuropeptides (Fuxe et al. 1976, 1977; Sarkar et al. 1981). The neuropeptides in the median eminence may be transported to the pituitary, or liberated into the cerebrospinal fluid (CSF) of the third ventricle or the subarachnoid space (Knigge et al. 1976; Bergland and Page 1978). In addition, the peptides can be transported back into the brain by a retrograde capillary flow from the pituitary to the median eminence—brain, or via a retrograde axonal transport (Oliver et al. 1977; Dorsa et al. 1979; Palkovits 1982). The possibility that a substance liberated into the CSF from the median eminence could be involved in the production of spasm, led us to examine CSF from both humans and rats with a SAH. We found a hitherto unknown spasmogenic compound which probably is a peptide. The compound appears at the same position in the chromatogram (high performance liquid chromatography) of the CSF from both humans and rats with a SAH (to be published). The substance is not present in CSF from SAH rats with lesions of the median eminence or the ascending NA pathways from the lower brain stem (to be published). It is not known if a humoral mechanism alone underlies the development of vasospasm post SAH, or if also a neural mechanism is involved. In this context it is noteworthy that Iadecola et al. (1983) have found that unilateral stimulation of the fastigial nucleus in normal rats produced a global vasodilatation that could be abolished in the frontal cortex ipsilateral to a basal forebrain lesion.

In conclusion: Our study has shown that the median eminence is essential in the development of vasospasm post SAH. If the median eminence produces vasospasm via a humoral, neural or a combined neural-humoral mechanism remains to be investigated.

ACKNOWLEDGEMENT

Supported by grants from the Einar Björkelund Foundation; Anna-Lisa and Sven-Eric Lundgren Foundation; Medical Faculty, University of Lund; Swedish Medical Association and the Swedish Medical Research Council (NO 04X-5300 and 14X-732).

The authors thank Dr. Jörgen Warberg, The Panum Institute, University of Copenhagen, Copenhagen, Denmark for the analysis of plasma prolactin, and Kerstin Stureson for preparation of histological sections.

REFERENCES

- Alper RH, Demarest KT, Moore KE (1980) Effects of surgical sympathectomy on catecholamine concentrations in the posterior pituitary of the rat. *Experientia* 36:134-135
- Baumgarten HG, Björklund A, Holstein AF, Nobin A (1972) Organization and ultrastructural identification of the catecholamine nerve terminals in the neural lobe and pars intermedia of the rat pituitary. *Z Zellforsch* 126:483-517
- Bergland RM, Page RB (1978) Can the pituitary secrete directly to the brain? (Affirmative anatomical evidence). *Endocrinology* 102:1325-1338
- Bishop W, Fawcett CP, Krulich L, McCann SM (1972) Acute and chronic effects of hypothalamic lesions on the release of FSH, LH and prolactin in intact and castrated rats. *Endocrinology* 91:643-656
- Björklund A, Falck B, Hromek F, Owman Ch, West KA (1970) Identification and terminal distribution of the tubero-hypophyseal monoamine fiber systems in the rat by means of stereotaxic and microspectrofluorimetric techniques. *Brain Res* 17:1-23
- Björklund A, Moore RY, Nobin A, Stenevi U (1973) The organization of tubero-hypophyseal and reticulo-infundibular catecholamine neuron systems in the rat brain. *Brain Res* 51:171-191
- Brizze KR, Palazzo MC, Klara PM, Hofer H (1978) Effects of systemic administration of 6-hydroxydopamine on the circumventricular organs in nonhuman primates. I. Area postrema. *Cell Tiss Res* 187:115-127
- de Champlain J (1971) Degeneration and regrowth of adrenergic nerve fibers in the rat peripheral tissues after 6-hydroxydopamine. *Can J Physiol Pharmacol* 49:345-355
- de Champlain J, Gauthier P, Porlier G, van Ameringen MR, Nadeau RA (1975) Compensatory mechanisms following peripheral chemical sympathectomy. In *Chemical Tools in Catecholamine Research, Vol 1* (eds G Jonsson, T Malmfors, Ch Sachs), Amsterdam, North-Holland, pp. 231-237

- Cruickshank JM, Neil-Dwyer G, Brice J (1974) Electrocardiographic changes and their prognostic significance in subarachnoid haemorrhage. *J Neurol Neurosurg Psychiatry* 37:755-759
- Cuello AC, Shoemaker WJ, Ganong WF (1974) Effect of 6-hydroxydopamine on hypothalamic norepinephrine and dopamine content, ultrastructure of the median eminence, and plasma corticosterone. *Brain Res* 78:57-69
- Day TA, Willoughby JO (1980) Noradrenergic afferents to median eminence: inhibitory role in rhythmic growth hormone secretion. *Brain Res* 202:335-345
- Delgado TJ, Brismar J, Svendgaard NAa (1985) Subarachnoid haemorrhage in the rat: angiography and fluorescence microscopy of the major cerebral arteries. *Stroke* 16:595-602
- Demarest KT, Moore KE (1979) Lack of a high affinity transport system for dopamine in the median eminence and posterior pituitary. *Brain Res* 171:545-551
- Dorsa DM, de Kloet ER, Mezey E, De Wied D (1979) Pituitary-brain transport of neurotensin: functional significance of retrograde transport. *Endocrinology* 104:1663-1666
- Finch L, Haeusler G, Kuhn H, Thoenen H (1973) Rapid recovery of vascular adrenergic nerves in the rat after chemical sympathectomy with 6-hydroxydopamine. *Brit J Pharmacol* 48:59-72
- Fuxe K, Hökfelt T, Agnati L, Löfström A, Everitt BJ, Johansson O, Jonsson G, Wuttke W, Goldstein M (1976) Role of monoamines in the control of gonadotrophin secretion. In *Neuroendocrine Regulation of Fertility* (ed. A Kumar), Basel, Karger, pp. 124-140
- Fuxe K, Löfström A, Agnati L, Eneroth P, Gustafsson JA, Hökfelt T, Skett P (1977) Central monoaminergic pathways. Their role in control of lutropin, follitropin and prolactin secretion. In *Endocrinology*, Vol 1 (ed. VHT James), Amsterdam, Excerpta Medica, pp. 136-143
- Gabrielsen TO, Greitz T (1970) Normal size of the internal carotid, middle cerebral and anterior cerebral arteries. *Acta Radiol (Diagn)* 10:1-10
- Gallardo E, Chiochio SR, Tramezzani JH (1984) Sympathetic innervation of the median eminence. *Brain Res* 290:333-335
- Hammermeister KE, Reichenbach DD (1969) QRS changes, pulmonary edema, and myocardial necrosis associated with subarachnoid hemorrhage. *Am Heart J* 78:94-100
- Hansen S, Stanfield EJ, Everitt BJ (1981) The effects of lesions of lateral tegmental noradrenergic neurons on components of sexual behavior and pseudopregnancy in female rats. *Neuroscience* 6:1105-1117
- Hawkins WE, Clower BR (1971) Myocardial damage after head trauma and simulated intracranial haemorrhage in mice: the role of the autonomic nervous system. *Cardiovasc Res* 5:524-529
- Iadecola C, Mraovitch S, Meeley MP, Reis DJ (1983) Lesions of the basal forebrain in rat selectively impair the cortical vasodilation elicited from cerebellar fastigial nucleus. *Brain Res* 279:41-52
- Iversen SD (1984) Cortical monoamines and behavior. In *Monoamine Innervation of Cerebral Cortex* (eds. L Descarries, TR Reader, HH Jasper), New York, Alan R Liss, pp. 321-349

- Jonsson G, Fuxe K, Hökfelt T (1972) On the catecholamine innervation of the hypothalamus with special reference to the median eminence. *Brain Res* 40:271-281
- Knigge KM, Joseph SA, Sladek JR, Notter MF, Morris M, Sundberg DK, Holzwarth MA, Hoffman GE, O'Brien L (1976) Uptake and transport activity of the median eminence of the hypothalamus. *Int Rev Cytol* 45:383-408
- Kågström E, Nilsson PE, Svendgaard NA (1969) Clinical and experimental spasm of the cerebral vessels. *Excerpta Med Int Congr Series* 193:60
- Lacy PS, Earle AM (1983) A small animal model for electrocardiographic abnormalities observed after an experimental subarachnoid hemorrhage. *Stroke* 14:371-377
- Laverty R, Sharman DF, Vogt M (1965) Action of 2,4,5-trihydroxyphenylethylamine on the storage and release of noradrenaline. *Br J Pharmacol* 24:549-560
- Lim AT, Smith GC, Clements JA, Funder JW (1984) Stress, dopaminergic blockade and median eminence – neurointermediate lobe catecholamine depletion: effects on hypothalamic, pituitary and plasma immunoreactive B-endorphin. *Clin Exp Pharmacol Physiol* 11:221-229
- Lindvall O, Björklund A (1983) Dopamine- and norepinephrine-containing systems: their anatomy in the rat brain. In *Chemical Neuroanatomy* (ed. PC Emson), New York, Raven Press, pp. 229-255
- Löfström A, Jonsson G, Fuxe K (1976) Microfluorometric quantitation of catecholamine fluorescence in rat median eminence. I. Aspects on the distribution of dopamine and noradrenaline nerve terminals. *J Histochem Cytochem* 24:415-429
- Macpherson P, Graham DI (1978) Correlation between angiographic findings and the ischemia of head injury. *J Neurol Neurosurg Psychiatry* 41:122-127
- Malmfors T (1971) The effects of 6-hydroxydopamine on the adrenergic nerves as revealed by the fluorescence histochemical method. In *6-Hydroxydopamine and Catecholamine Neurons* (eds. T Malmfors, H Thoenen), Amsterdam, North-Holland, pp. 47-58
- Malmfors T, Sachs Ch (1968) Degeneration of adrenergic nerves produced by 6-hydroxydopamine. *Eur J Pharmacol* 3:89-92
- McCann SM, Krulich L, Ojeda SR, Negro-Vilar A, Vijayan E (1979) Neurotransmitters in the control of anterior pituitary function. In *Central Regulation of the Endocrine System* (eds K Fuxe, T Hökfelt, R Luft), New York, Plenum Press, pp. 329-347
- Neil-Dwyer G, Cruickshank J, Stott A, Brice JC (1974) The urinary catecholamine and plasma cortisol levels in patients with subarachnoid haemorrhage. *J Neurolog Sci* 22:375-382
- Offerhaus L, Van Gool J (1969) Electrocardiographic changes and tissue catecholamines in experimental subarachnoid haemorrhage. *Cardiovasc Res* 3:433-440
- Oliver C, Mical RS, Porter JC (1977) Hypothalamic-pituitary vasculature: evidence for retrograde blood flow in the pituitary stalk. *Endocrinology* 101:598-604
- Palazzo MC, Brizzee KR, Hofer H, Mehler WR (1978) Effects of systemic administration of 6-hydroxydopamine on the circumventricular organs in nonhu-

- man primates. II. Subfornical organ. *Cell Tiss Res* 191:141-150
- Palkovits M, Léránth CS, Zabórsky L, Brownstein MJ (1977) Electron microscopic evidence of direct neural connection from the lower brain stem to the median eminence. *Brain Res* 136:339-344
- Palkovits M (1982) Neuropeptides in the median eminence: their sources and destinations. *Peptides* 3:299-303
- Peerless SJ (1980) Postoperative cerebral vasospasm without subarachnoid hemorrhage. In *Cerebral Arterial Spasm: Proceedings of the Second International Workshop*, Amsterdam (ed. RH Wilkins), Baltimore/London, Williams & Wilkins, pp. 496-498
- Porter JC, Smith KR (1967) Collection of hypophysial stalk blood in rats. *Endocrinology* 81:1182-1185
- Saavedra JM (1985) Central and peripheral catecholamine innervation of the rat intermediate and posterior pituitary lobes. *Neuroendocrinology* 40:281-284
- Sachs Ch, Jonsson G (1975) Mechanisms of action of 6-hydroxydopamine. *Biochem Pharmacol* 24:1-8
- Sarkar DK, Smith GC, Fink G (1981) Effect of manipulating central catecholamines on puberty and the surge of luteinizing hormone and gonadotropin releasing hormone induced by pregnant mare serum gonadotropin in female rats. *Brain Res* 213:335-349
- Sawchenko PE, Swanson LW (1981) Central noradrenergic pathways for the integration of hypothalamic neuroendocrine and autonomic responses. *Science* 214:685-687
- Simeone FA, Peerless SJ (1975) Prolonged cerebral vasospasm without subarachnoid hemorrhage. In *Subarachnoid Hemorrhage and Cerebrovascular Spasm* (eds. RR Smith, JT Robertson), Springfield, Charles C Thomas, pp. 206-225
- Smith GC, Helme RD (1974) Ultrastructural and fluorescence histochemical studies on the effects of 6-hydroxydopamine on the rat median eminence. *Cell Tiss Res* 152:493-512
- Smith GC, Courtney PG, Wreford NGM, Walker MMCD (1982a) Further studies on the effects of intravenously administered 6-hydroxydopamine on the median eminence of the rat. *Brain Res* 234:101-110
- Smith GC, Sheward WJ, Fink G (1982b) Effect of 6-hydroxydopamine lesions of the median eminence and neurointermediate lobe on the secretion of pituitary hormones in the male rat. *Brain Res* 246:330-333
- Suwanwela C, Suwanwela N (1972) Intracranial arterial narrowing and spasm in acute head injury. *J Neurosurg* 36:314-323
- Svendgaard NAa, Brismar J, Delgado TJ, Rosengren E (1985a) Subarachnoid haemorrhage in the rat: effect on the development of vasospasm of selective lesions of the catecholamine systems in the lower brain stem. *Stroke* 16:602-608
- Svendgaard NAa, Brismar J, Delgado TJ, Diemer NH (1985b) The effect on the development of cerebral vasospasm in the rat of lesioning of the peripheral and central catecholamine systems. *Neurol Res* 7:30-34
- Svendgaard NAa, Delgado TJ, Rosengren E (1986) Effect of selective lesions of medullary catecholamine nuclei on experimental cerebral vasospasm in the rat. Submitted for publication

- Tranzer JP, Thoenen HC (1967) Ultramorphologische Veränderungen der Sympathischen Nervenendigungen der Katze nach Vorbehandlung mit 5- und 6-Hydroxydopamine. Naunyn Schmiedebergs Arch Pharmac Exp Path 257:343-344
- Weisenberg LS, De Nicola AF, Arakelian MC, Libertun C (1979) Effect of median eminence lesions on ^3H estradiol binding in the anterior pituitary and hypothalamus. Endocrinology 105:1152-1157
- Wilkins RH, Odom GL (1970) Intracranial arterial spasm associated with craniocerebral trauma. J Neurosurg 32:626-633
- Wilkins RH (1975) Hypothalamic dysfunction and intracranial arterial spasms. Surg Neurol 4:472-480
- Wilson JL, Feild JR (1974) The production of intracranial vascular spasm by hypothalamic extract. J Neurosurg 40:473-479
- Wood JH (1982) Neuroendocrinology of cerebrospinal fluid: peptides, steroids, and other hormones. Neurosurgery 11:293-305

